1

Alliesthesia in visual and auditory sensations from environmental signals

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Introduction: 'Alliesthesia' describes the fact that sensory stimuli can arouse pleasant or unpleasant sensations according to the internal state of a person. In the present work, we tested the hypothesis that the hedonicity of visual and auditory sensations aroused by the environment might be more or less intense and could even reverse valence according to the participant's internal state.

Methods: Fifteen healthy participants (8 men, 7 women, 21 ± 4 yr) took part in 5 experimental sessions. During all sessions, participants stayed in an 11.6 m^2 quiet blind room, with white walls, and without decoration or furnishing. Sessions differed from one to the other by the visual and auditory environments proposed and by the time of day when the measurements were made (daytime or middle of the night). The 5 sessions were as follow: 1) daytime without sensory stimulations (no video–tape); 2) daytime with poor sensory stimulations (uninteresting video–tape film); 3) daytime with rich sensory stimulations (interesting chosen movie on video–tape); 4) night-time without sensory stimulations (no video–tape); 5) night-time with poor sensory stimulations (uninteresting video–tape). Several magnitude ratings of the participant's sensations and motivations were measured by visual analogical scales as, 'Tiredness', 'Desire to leave the environment' and 'Hedonicity aroused by the environment'.

Results: During the day, hedonic ratings decreased in the no- and uninteresting video–tape film conditions ($P < 0.01$), but remained stable with the interesting chosen movie. During the night, hedonic ratings decreased similarly to daytime ratings with the uninteresting video–tape film ($P < 0.01$) but rose in the no-video–tape environment ($P < 0.01$). The time course of motivation to leave the environment mirrored that of hedonic ratings. Changes in hedonic ratings and motivation to leave the environment in the day-no-video and night-no-video situations correlated ($r = 0.541$ and $r = -0.593$; $P < 0.01$) with the state of tiredness.

Conclusion: Alliesthesia occurred in visual and auditory sensations that originated from the environment, and motivated behaviours that were not consumatory. Such results suggest that alliesthesia is a general property of all sensations, and emphasizes the fundamental role of pleasure in motivation for all behaviours.

2

Electrophysiological correlates of metabolic abnormalities during interictal state in temporal lobe epilepsy

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Introduction: Magnetic resonance spectroscopic imaging (MRSI) is a technique allowing quantification of brain metabolites. Recently, it has been shown that the decrease of the relative concentration of N-acetyl-aspartate (NAA), a neuronal marker, was linked to functional alterations rather than neuronal loss in partial epilepsies (1,2). However, the exact functional correlates of such changes are still poorly understood. In this study we aimed at demonstrating a correlation between such alterations and interictal electrophysiological abnormalities in temporal lobe epilepsy (TLE).

Methods: We studied 20 patients suffering from drug-resistant TLE enrolled in a presurgical evaluation and benefiting from a depth electrodes recording (stereo-electroencephalography, SEEG). We performed an MRSI protocol before SEEG, allowing correlation of metabolic and electrical abnormalities in five standardized regions of interest (ROI): two mesial temporal areas and three neocortical areas. Relative concentration of NAA was then compared to the rate of interictal spikes recorded by SEEG, using a Spearmann's rank test.

Results: Considering all ROIs, we found a correlation between the decrease of the relative concentration of NAA and the rate of interictal spikes ($P < 0.0001$). This correlation was also observed when considering either mesial ROIs ($P = 0.002$) or neocortical ROIs ($P = 0.03$).

Conclusion: This study is the first demonstration of a correlation between NAA decrease and interictal electrophysiological abnormalities in mesial as well as in neocortical areas. This suggests a link between mitochondrial metabolism (which may be reflected by NAA) and interictal spikes in TLE. In addition, MRSI may be helpful in clinical practice, by defining non-invasively the zones involved by interictal electrophysiological alterations.

3

Synthesis of a new serotonergic drug. A behavioural and neurochemical study in mice

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Introduction: Serotonergic neurotransmission is widely involved in many neurologic disorders such as anxiety, depression, and addiction. For this reason, 5-HT1 receptors are often major targets of drugs designed for the above mentioned diseases. Despite a significant progress in the pharmacology of neuroleptic drugs, side effects such as somnolence, vigilance decrease, addiction, and enzymatic induction remain a concern. The aim of the present investigation is to find a new neuroleptic drug devoid of side effects.

Methods: A drug was synthesized and was intraperitoneally injected to Swiss mice at the dose of 30 mg/kg. For neurochemical studies, the animals were decapitated and their heads were frozen in liquid nitrogen. The frozen encephalons were dissected out and homogenized. The homogenates were centrifuged and the concentrations of serotonin, its precursor tryptophan, and its principal metabolite 5-hydroxyindole acetic acid (5-HIAA) were determined by high performance liquid chromatography in the supernatant.

Results: By chemical synthesis, we obtained 8- {4- [(6-Methoxy-2,3-dihydro-[1,4] dioxino [2,3-b] pyridin-3-ylmethyl) -amino] -butyl} -8-aza-spiro [4,5] decane-7,9dione from a pyridine derivative 5-bromo-2-methoxypyridine. This molecule was termed JB788 and was synthesized in seven steps with a global yield of 10.4%. We showed that JB788 presents a strong affinity for 5-HT1A receptors ($Ki = 6.10^{-11}$ M). By behavioural studies, we observed that JB788 induced a decrease in the spontaneous activity in mice. However, it did not alter the animal reactivity to various stimuli. The behavioural observations were also the same when the JB788 dose was lowered up to 5 mg/kg. Moreover, this drug largely decrease the animal aggressiveness when either 5 mg/kg or when 30 mg/kg were used. Neurochemical studies showed that tryptophan concentration increased 30 min after the administration of 30 mg/kg of JB788 in cerebral cortex (55 ± 7 pmol/mg protein versus 142 ± 22 pmol/mg protein; means ± SEM). This increase was transient since the tryptophan concentration became normal 1 h after dosing. Serotonin concentration lately decreased 24 h after dosing in different regions of the encephalon. No change was observed in 5-HIAA concentration in cerebral cortex.

Conclusion: These results show that change in tryptophan concentration is not parallel to change in serotonin concentration and so, JB788 may also interact with other metabolic pathways. Whatever, JB788 is the most powerful ligand of 5-HT1A receptors known to date and it displays neuroleptic activities in mice probably by interacting with indoleaminergic system.

4

TREK-1, a K^+ channel involved in polymodal pain

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Introduction: Ion channels play a very important role in the detection of pain. The TREK-1 channel is a member of the 2P-domain K^+ channel (K_{2p}) family, and it was first mammalian mechanosensitive K^+ channel to be identified at molecular level. It is present in the peripheral sensory system, particularly in small dorsal root ganglion (DRG) neurons that are associated with nociception. This channel also has a steep temperature sensitivity between ~15 and ~40°C, making it a candidate for detection of cold and/or heat in addition to mechanical stimuli. The objective of this work is: (1) to examine the exact the localization of channels TREK-1 in different types of sensory neurons (2) to make use of TREK-1 knockout mice to evaluate the exact role of this temperature, mechano- and osmo-sensitive K^+ channel in pain perception associated with different types of stimuli.

Methods: Mice with a disrupted TREK-1 gene were used in order to study their reaction after application of various stimuli.

Results: TREK-1 is highly expressed in small sensory neurons, is present in both peptidergic and non-peptidergic neurons and extensively colocalized with TRPV1. Mice with a disrupted TREK-1 gene are more sensitive to pain heat sensations near the threshold between anoxious warmth and painful heat. Knockout animals also display an increased thermal and mechanical hyperalgesia in conditions of inflammation proving the important role for TREK-1 in polymodal pain perception. They display a largely decreased response to hypotonicity induced nociception in prostaglandin E2 sensitized animals.

Conclusion: The TREK-1 appears as an important ion channel for polymodal pain perception and as an attractive target for the development of new analgesics.

5

Effects of the blockade of the 5-HT6 serotoninergic receptors on scopolamine-induced memory deficits in mice

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Introduction: Involvement of 5-HT6 serotoninergic receptors (5-HT6R) in memory and learning processes has been addressed mainly in the rat and to a lesser extent in the mouse. Indeed, we have demonstrated that the blockade of 5-HT6R improved spatial recognition memory (acquisition and consolidation), but not reference memory performances of mice. Here, we chose to assess in the mouse the effects of the blockade of 5-HT6R on acquisition, consolidation and retention performances in working memory and in long term memory through the use of the scopolamine-induced deficit model.

Methods: With this aim, we have studied the effects of the selective 5-HT6R antagonist, SB-271046 (3, 10, 30 mg/kg, i.p.), on the learning impairment induced by scopolamine (1 mg/kg, s.c.) in mice. Working memory has been assessed through the spontaneous alternation task in the Y-maze and long term memory has been studied in the passive avoidance paradigm (Session 1: training; session 2: test; inter-trial interval: 24 h).

Results: Our results demonstrate that SB-271046 (3 and 30 mg/kg) reversed the scopolamine-induced deficits in working memory of mice. When given alone at the same doses, the antagonist failed to exhibit any measurable effects. In the passive avoidance task, SB-271046, given 1 h prior to training, tends to reverse the scopolamine-induced deficit in acquisition. Similarly, SB-271046, given 1 h before session 2, significantly reversed the mnemonic deficit caused by scopolamine administered 30 min before session 2 (retention). Both effects were dose-dependent in nature over a range of 3-30 mg/kg. In contrast, SB-271046, administered just after session 1, failed to reverse the scopolamine-induced deficits (consolidation).

Conclusion: We thus hypothesize that mouse 5-HT6R are, as in the rat, implicated in the regulation of the cholinergic neurotransmission and that such an implication could find an application in the field of studies dedicated to the search for treatments of memory alterations associated to ageing.

6

Effect of chronic ingestion of methionine on extracellular matrix proteins

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Introduction: Several studies have shown that an excess of methionine induces an hyperhomocysteinemia state and a multiorgan damage. In this work, the effect of a chronic administration of methionine on proteins extracellular matrix levels is study on rats laboratory *Rattus norvegicus*.

Methods: Animals intake the methionine in drink water with a quantity of 200 mg/Kg of body weight/day during 6 months. Body weight and plasmatic levels of glucose, cholesterol, triglycerids and urea are regulary determined. Kidney, adrenal and pancreas removed at the end of experiment are fixed and embedded in paraffine. Collagenous and non collagenous proteins are assayed with spectrophotometric method applied to histologicals preparations stained with sirius red and fast green.

Results: Our results don't show significant differences between control and experimental animals body and organ (kidney, adrenal) weight's and plasmatic parameter's. Colometric assay of extracellular matrix proteins report that methionine intake increases collagenous in pancreas and a non collagenous proteins in adrenal. Hence, methionine seem decrease non collagenous proteins in pancreas. Any significant effect is enregistred in kidney.

Conclusion: This results show that chronic administration of methionine modify extracellular matrix turn over at a specific tissue manner.

7

DHA modulates Ca^{2+} signaling through the phosphorylation of tyrosine kinases in human Jurkat-T cells

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Introduction: Epidemiological, clinical and experimental studies have established that n-3 polyunsaturated fatty acids, especially docosahexaenoic acid (DHA), exert beneficial effects in several autoimmune and inflammatory disorders. However, its molecular mechanisms of action are still poorly understood. T-cell receptor activation leads to the activation of $PLC^{\gamma 1}$and the production of IP_3, and DAG. Binding of IP_3 to its receptor on endoplasmic reticulum (ER) releases Ca^{2+} from the stores that is followed by Ca^{2+} influx across the plasma membrane, which is termed stoe-operated Ca^{2+} (SOC) entry. Several studies have shown that PTK can regulate calcium channels activities in several cell types. Here, we investigated the effects of DHA on calcium signaling and the implication of PTK in this pathway in Jurkat T cell line.

Methods: The concentration in free intracellular Ca^{2+} ($[Ca^{2+}]_i$) was measured by the fura 2/AM probe. Phosporylation of tyrosine kinases was assessed by using specific anti-PTK antibodies and was determined by western blotting.

Results: DHA induced dose-dependent increases in $[Ca^{2+}]_i$ which were contributed by intracellular pool and store-operated calcium (SOC) influx via opening of Ca^{2+} release-activated Ca^{2+} (CRAC) channels in Jurkat T cells. Pre-incubation of Jurkat T cells with PP2, a selective inhibitor of $p56^{lck}$, $p59^{fynT}$ and Hck PTK, as well as SU6656, a specific inhibitor of $p59^{fynT}$, Yes and Lyn PTK, significantly potentiated DHA-induced rises in $[Ca^{2+}]_i$. In Ca^{2+}-free Ca^{2+} reintroduction (CFCR) experiments, we have shown that PTK inhibition by PP2 or SU6656, significantly enhanced Ca^{2+} influx without affecting intracellular Ca^{2+} pool mobilisation induced by this fatty acid, thus indicating that PTK are implicated in the opening of CRAC channels in these cells. To gain insight into the identity of the PTK implicated in DHA-induced response, we used a Jurkat T cell line deficient in the $p56^{lck}$ PTK (JCam 1.6). We observed that DHA-induced rises in $[Ca^{2+}]_i$ were not altered in JCam 1.6 thus suggesting that $p56^{lck}$ is not the PTK implicated, but rather $p59^{fynT}$. Implication of $p59^{fynT}$ PTK was further supported by immunoprecipitation experiments showing that DHA induced $p59^{fynT}$ phosphorylation, which was found to be potentiated by either PP2 or SU6656.

Conclusion: The present study shows that DHA stimulates Ca^{2+} influx through the activation of CRAC channels and $p59^{fynT}$ activation in Jurkat T cell line.

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Metabolic endotoxemia initiates obesity and insulin resistance

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Introduction: Diabetes and obesity are two metabolic diseases characterized by insulin resistance and a low grade inflammation. Seeking an inflammatory factor causative of the onset of insulin resistance, obesity, and diabetes, we identified bacterial lipopolysaccharide (LPS) as a triggering factor.

Methods: We found that normal endotoxemia increases or decreases during the fed or fasted state on a nutritional basis, respectively; and that a four-week HF feeding, which induced obesity and diabetes, chronically increased plasma LPS concentration 2–3 times, a threshold that we defined as 'metabolic endotoxemia'.

Results: When metabolic endotoxemia was induced for four weeks in normal chow-fed mice by continuous subcutaneous infusion of LPS, fasted glycemia, insulinemia, whole body, liver, and adipose tissue weight gain and markers of inflammation in the liver, adipose depots, and muscle were all increased and to a similar extent as HF mice. Furthermore, liver but not whole body insulin resistance was detected in LPS-infused mice. CD14 mutant mice resisted LPS-induced body, liver, and adipose weight gain and diabetes. This second new finding demonstrates that metabolic endotoxemia dysregulates the inflammatory tone and triggers body weight gain and diabetes. Then we found that CD14 mutant mice were hypersensitive to insulin. Hence, we challenged the mutant mice by a HF diet for a long term and found that the occurrence of insulin resistance, glucose intolerance, and body weight gain were delayed by several months.

Conclusion: All these findings allow concluding that LPS/CD14 system sets the tone of insulin sensitivity, and the onset of diabetes and obesity. In conclusion, lowering the plasma LPS concentration would be a potent strategy for the control of metabolic diseases.

9

Regulatory T cell functions are regulated by a dietary fatty acid, docosahexaenoic acid (DHA), in wild type and PPARα-null mice

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Introduction: CD4+CD25+ regulatory T (T-reg) cells are essential for the induction and maintenance of immunologic self-tolerance as well as transplant tolerance, and several models support the idea of the peripheral generation of CD4+CD25+ T-reg from CD4+CD25- T cells. However, little is known about the endogenous factors and mechanisms controlling their suppressive capacity on immune response.

Methods: Responder CD4+CD25- T cells or T-reg cells were pre-incubated with DHA. Then, CD4+CD25- T cells were cultured in presence or absence of T-reg cells.

Results: We showed that DHA curtailed the *in vitro* inhibitory function of mice purified T-reg cells, in a dose-dependent manner. This suppressive capacity of T-reg cells was weaker in peroxisome proliferator-activated receptor-alpha (PPARα) -null mice than in wild type (WT) mice. However, the index on anti-CD3-stimulated T cell proliferation was higher in WT mice (3.3 fold) than in PPARα-null mice (2.1 fold). In addition, the inhibitory action of T-reg was also low when CD4+ T responder cells were pre-incubated with DHA. DHA induced the expression of a wide range of genes including forkhead/winged helix transcription factor (FOXP3), cytotoxic T lymphocyte-associated antigen (CTLA)-4 and transforming growth factor (TGF) -β in T-reg cells from WT mice, whereas it decreased their expression in T-reg cells from PPARα-null mice. Moreover, DHA exposure induced a regulatory phenotype in CD4+CD25- T cells by inducing the expression of T-reg cell-specific FOXP3 gene only in WT mice.

Conclusion: These results suggest that PPARα may be implicated in the suppressive capacity of T-reg cells and the DHA reverses their inhibitory action on T cell proliferation.

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Morphological regionalization and multidimensional analysis effects of castration and efferent duct ligation on the proximal epididymis of sand rat *Psammomys obesus* Cretzschmar, 1828

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Introduction: In the mammals, the epididymis duct is not a simple channel of transport and storage of the spermatozoa but a true key body of postgonadic maturation. This maturation is conditioned by an environment created by the cells which constitute its epithelium.

Methods: Animals: The sand rat is diurnal rodent which lives in the Sahara desert near wadis; adult animals were trapped in the wild in the region of Béni Abbès (Algeria). In the autumn (breeding season), adult males castrated and 30 days later some were killed, whilst others were injected with testosterone oenanthate twice daily (75 µg/injection) for 15 days. The last group is that of the animals having undergone the efferent duct ligation.

Histology: Statistical analysis: Comparisons between regions were calculated using analysis the variance (ANOVA). The repercussion study of experimentation on the cellular morphometry has necessity the principal component analysis (ACP).

Results: In spite of the morphological resemblance of the principal cells which constitute the epithelium, the morphometric study of the proximal epididymis showed the existence five regions statistically different.

The principal component analysis (ACP) of the castration effects, testosterone treatment after castration and the efferent duct ligation on all principal cells dimensions showed that the epithelial height and supranuclear area are affected by these experiments.

The experiments of castration showed structural damage and a very significant intertubular proliferation. These effects are partially restored by testosterone injection. The experiments of efferent duct ligation induce epithelial height reduction and cells extrusion in the tubular lumen.

Conclusion: The correlation between these results makes it possible to classify these two parameters as principal actors for the exploration of the androgens and/or the testicular factors deprivation effects on cells morphology.

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Preliminary characterization and immunohistochemical localization of POSVP21 in the sand rat (*Psammomys obesus*) seminal vesicles

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Introduction: A major secretory protein band (M.W. 21 000) regulated by testosterone was resolved by SDS-PAGE from sand rat seminal vesicles during breeding season. In conclusion, a major androgen-dependent protein was demonstrated in mature sand rat seminal vesicles.

Methods: When analyzed by NepHGE the protein band of 21 000 appeared to be composed of at least three visible spots with pHi values varying from 4 to 7.

Polyclonal antibodies against $POSVP_{21}$, were raised in rabbits, selected according to their capacity to specifically recognize the protein and purified by affinity chromatography.

The 21 kDa designed as $POSVP_{21}$ (*Psammomys obesus* seminal vesicles protein 21 kDa) has been purified in high yield from polyacrylamide gels using electro elution. Polyclonal antibodies were also used to study immunohistochemical antigen localization by the avidin-biotin peroxidase procedure.

Immunohistochemical staining using the Polyclonal antibodies specific for $POSVP_{21}$was performed to localize the protein in histological sections of different parts of the seminal vesicles.

Results: Quantitatively, the 21 kDa protein synthesized in large amounts when the androgen level increases, and accounts for over 22% of soluble proteins from homogenate of seminal vesicles during breeding season. Its partially internal sequence was identified and exhibits five peptides.

The secretory epithelium of seminal vesicle showed a strong immunohistochemical reaction by indirect peroxidase staining. The distribution of $POSVP_{21}$ in various sections of seminal vesicles was analyzed and observation showed that it is localized in the cytoplasm of epithelial cells and in secretory products in the lumen. The connective tissues and nucleus had negative immunoreactions for $POSVP_{21}$.

Total RNA from seminal vesicles was translated in a cell-free system derived from rabbit reticulocyte lysate and ^{35}S methionine. Two major bands of M_r 14 400 and 21 000 were visualized by denaturing gel electrophoresis.
Conclusion: In conclusion, a major androgen-dependent protein was demonstrated in mature sand rat seminal vesicles.
The preliminary characterization of $POSVP_{21}$ show's that it is primarily: an intra cellular and cytoplasmic protein.

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Mitochondrial T3 receptors define a new endocrine pathway
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Introduction: Numerous data have brought strong evidences that hormonal activity is hormonally regulated in particular by thyroid hormone. However, despite the occurrence of short-term influences also observed in isolated organelles, the existence of a direct mitochondrial T3 pathway has raised important controversies. Therefore, the purpose of this long term study was to characterize this pathway.
Methods: With this aim, we first identified mitochondrial T3 receptors using photoaffinity labelling and western blot experiments. Thereafter, we studied the mitochondrial import of these receptors and their intramitochondrial mechanisms using in organello import or transcription experiments. Last, we characterized some important nuclear genes targetted by this pathway.
Results: Using photoaffinity labelling experiments with highly purified rat liver organelles, we demonstrated that two mitochondrial proteins with molecular mass of 43 and 28 kDa specifically bind T3. In addition, western blot experiments with two different antibodies raised against the nuclear T3 receptor TRα1 led to the conclusion that these proteins are N-terminally truncated forms of this receptor. We provided evidence that they are synthesized through the use of internal AUGs occurring in the transcript encoding TRα1 and that they are specifically addressed to mitochondria. Whereas p28 is located in the inner membrane, p43 is located in the mitochondrial matrix (1).
Study of the mitochondrial import of these receptors demonstrated that it occurs according to an atypical process involving two mitochondrial import sequences located in the C-terminal part of the proteins and a N-terminal permissive sequence not occurring in TRα1, and responsible of the different cellular localizations of TRα1, p43 and p28.
Whereas the function of p28 remains to be elucidated, we demonstrated that p43 heterodimerizes with two other truncated forms of nuclear receptors (mt-PPAR and mt-RXR), binds to specific sequences of the mitochondrial genome and stimulates the expression of mitochondrial genes. This leads to a stimulation of mitochondrial protein synthesis, respiratory chain activity and mitochondriogenesis (1, 2).
In turn, this stimulation of mitochondrial activity influences the expression of important nuclear genes such as c-Myc, calcineurin and myogenin in myoblasts or tumour suppressor genes and proto-oncogenes in fibroblasts (3).
Conclusion: In conclusion, this work demonstrates the occurrence of a new endocrine pathway involving mitochondrial receptors regulating not only energy metabolism but also important cellular processes such as cell differentiation and oncogenesis.

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Simple and robust assessment of insulin sensitivity (SI) with the oral minimal model in diabetics
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Introduction: Despite the major pathophysiological relevance of insulin sensitivity (SI) this parameter is seldom measured, due to the complexity and the cost of reference methods (glucose clamp, minimal model analysis of an intravenous glucose tolerance test [IVGTT]). Recent studies have emphasized the accuracy of a simpler approach: the minimal model analysis of a standardized hyperglucidic breakfast, i.e. a 'physiological' procedure to assess glucose tolerance, that is also suitable for the diagnosis of reactive hypoglycemia [1]. We recently reported [2] that this 'oral minimal model' (OMM) provides a robust alternative to the more classical IVGTT-based procedure. In this study we focused on the validity of the OMM compared to IVGTT in diabetic patients.
Methods: Seventeen diabetics not treated by insulin (type 2 diabetics and atypical diabetes; sex ratio M/F: 7/10; age: 49.94 ± 0.78; weight: 78.54 ± 0.91; height: 165.32 ± 0.34; representing a wide range of body mass index (mean: 28.81 ± 0.35; range 18.7–39.5); and SI 2.42 ± 0.15 $min^{-1}/(\mu U/mL.\ 10^{-4})$; range: 0.2 à 9.1 $min^{-1}/(\mu U/ml.\ 10^{-4})$ underwent the two tests.
Results: Determinations of SI are correlated ($r = 0.577$) with a systematic difference of 4.18 ± 0.15 $min^{-1}/(\mu U/mL.\ 10^{-4})$ due to the already reported fact that the OMM almost never yields in 'SI-zero values', a particularity of the IVGTT procedure which has been often criticized. Actually if a systematic correction of 4.2 $min^{-1}/(\mu U/mL.\ 10^{-4})$ is applied the agreement between IVGTT and OMM is excellent on Bland-Altman plots (mean difference 0.176; range: −1.19 to 1.54). Therefore, if the cutoff for defining insulin resistance is shifted from 2 to 6.5 $min^{-1}/(\mu U/mL.\ 10^{-4})$ there is only one misclassification of patient with the OMM, i.e. on this sample the sensitivity of the technique is 91.7%, its specificity 100%, its positive predictive value 100%, its negative predictive value 85.7%. The prediction of glucose effectiveness S_G with the formula $S_G = 2.92 \exp^{(-0.18\ [G60-G0])}$ is also excellent ($r = 0.728$, mean difference 0.03 ± 0.03).
Conclusion: Thus the OMM provides a safe, cheaper and easier to perform alternative to the IVGTT and can be used to assess SI and S_G in diabetics.

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Sexual maturation in Tunisian children
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Introduction: Sexual maturation is dependent on genetic and environmental factors. The differences in the age of sexual maturation between populations are largely due to differences in socio-economic conditions, as well as genetic factors. Therefore, each ethnic group has to construct its own normative data.
The purpose of this study was to examine the sexual maturation in Tunisian children.
Methods: Six hundred and eighty-four Tunisian children (351 boys and 333 girls) between the age of 8 and 16 were selected. The children's pubertal stages were assessed using the composite score as described by Tanner JM (1962) for males and females by the same endocrinologist: breast development (B1-B5), pubic hair (PH1-PH5) and axillary hair (AH1-AH3) in girls; genitalia development (G1-G5), pubic hair (PH1-PH5) and axillary hair (AH1-AH3) in boys.
Analysis of variance (one way ANOVA) for pubertal stages (5 modalities) followed by a Scheffe post-test were applied to compare age and height according to pubertal stage. All analyses were performed using STAT VIEW Software version 5.0.
Results: A total of 178 males and 142 females were prepubescent (T1), 173 males and 154 females had a puberty stage included between 2 and 4 (T2: 89 males and 57 females, T3: 49 males and 57 females and T4: 35 males and 40 females). 37 females but no males have achieved their puberty (T5).
A large variation was observed in the distribution of children's age and height by pubertal stages in both sexes.
The Analysis of variance shows a significant increase of height with age and pubertal stage in both males and females.
In addition our results showed a large variation in timing of puberty depending on sex and individual development even among children of the same gender.
Conclusion: This study concerning this age group is important for clinical purposes. The pubertal status is crucially important when comparing patients, especially those with chronic diseases, to their healthy counterparts.

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Perinatal programming of adipose tissue micro-inflammatory syndrome and vascular risk factors in the rat
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Introduction: It is now well established that obesity and the metabolic syndrome are associated with a low grade inflammation, and metabolic and cardiovascular complications, possibly subsequent to alterations in deleterious adipokines secretion. Changes in perinatal environnement have been shown to raise metabolic syndrome prevalence and to induce increased adipose tissue glucocorticoid metabolism in adulthood. So, we analyzed the expression of adipose tissue pro-inflammatory cytokines and vascular risk factors, in a previously described model of perinatal programming of obesity and the metabolic syndrome.
Methods: Postnatal overfeeding (POF) was induced in the rat by a reduction of the litter size to three pups between P3 and P21. Control or POF rats were weaned at P21, then fed either with a standard diet (3.5 Kcal/g and 4% fat) or with a high fat diet (5 Kcal/g and 30% fat) until adulthood. We quantified in adult rats, using *in situ* hybridization, adipose tissue expression of the mRNAs coding for various cytokines as tumor necrosis α (TNFα), TNF receptor I (TNF-RI), and interleukin 6 (IL-6), and an important vascular risk factor, plasminogen activator inhibitor-1 (PAI-1).
Results: As compared to controls, POF rats fed with standard diet showed increased visceral adipose tissue (VAT) concentrations of the mRNAs coding for TNFα (3.76 ± 0.26 vs. 1.41 ± 0.18 nCi/g), TNF-RI (10.85 ± 0.72 vs. 4.51 ± 0.64 nCi/g), IL-6 (2.71 ± 0.36 vs. 1.25 ± 0.32 nCi/g) and PAI-1 (60.61 ± 3.06 vs. 51.14 ± 2.11 nCi/g) ($P < 0.01$). High fat diet induced increased VAT expression of the above-mentioned parameters, which were further stimulated in POF rats (TNFα: 4.97 ± 0.4 vs. 2.65 ± 0.73 nCi/g; TNF-RI: 11.73 ± 0.76 vs. 8 ± 1.72 nCi/g; IL-6: 4.16 ± 0.39 vs. 2.12 ± 0.11 nCi/g; PAI-1: 67.65 ± 1.75 vs. 58.91 ± 5.99 nCi/g) ($P < 0.01$).
Conclusion: Our results suggest that POF programs VAT micro-inflammation and increased vascular risk factors, comparable to those found in humans. The above-mentioned overexpression of pro-inflammatory cytokines could induce a counter-regulatory mechanism, such as increased expression of local glucocorticoid metabolism (11β hydroxysteroid dehydrogenase type 1 [11β-HSD-1] and glucocorticoid receptors), as previously shown. In addition we show that POF exacerbates VAT response to high fat feeding.

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Non linear analysis of the ventilatory behavior of the isolated brainstem of the frog, *Rana esculenta*
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Introduction: Human ventilation at rest seems cyclic and regular. Its dynamics is however chaotic, which means deterministic but non-linear, complex, sensitive to the initial conditions and unpredictable in the long term. The origin of this chaotic behavior is unknown but it could arise from the automatic breathing command located in the brainstem. Indeed, the central pattern generator encompasses several oscillators whose interactions could theoretically lead to a chaotic behavior. To test this hypothesis we have analyzed the neural ventilatory dynamics of the isolated brainstem of the tadpole, *Rana esculenta*. This *in vitro* preparation, devoid of any afferent input except central chemosensitivity, produces the gill/buccal and lung rhythms of the living animal. These two rhythms depend on two coupled oscillators.
Methods: The study was carried out on preparations from five pre-metamorphic (larval stages) and five post-metamorphic (near adult stages) tadpoles. The neurogram of the 7th cranial nerve was recorded during 2.2 to 5 minutes. The preparations were superfused with an artificial cerebro-spinal fluid of various pH: acidic (7.4 and 7.6), normal (7.8) and alkaline (8.0). The root mean square signals (RMS) of the raw neurograms were undersampled at 40 Hz. The chaotic behavior of the whole trajectory of the signals was assessed with the noise titration method, a reliable and robust technique to detect chaos in short and noisy time series.
Results: Whatever the pH and the developmental stage, the noise titration process did not detect any non-linearity in the signals. This means that the neural ventilation of the isolated brainstem of the tadpole follows a non-chaotic trajectory.
Conclusion: The neural ventilation of the isolated brainstem of the tadpole is not chaotic. The chaotic behavior of the ventilatory flow in humans could result either from a phylogenetic difference or from afferent inputs which influence ventilation *in vivo* like, for example, vagal afferents.

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Central role of NO in ventilatory acclimatisation to hypoxia in a model of anemic transgenic mice

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Introduction: Polycythemia and increase in ventilation are considered as important factors of acclimatisation to hypoxia. Despite low oxygen carrying capacity, it has been recently shown that anemic transgenic mice (Epo-Tag^h mice) adapt to chronic hypoxia partly through an increase in ventilatory acclimatisation (VAH). Nitric oxide (NO) has been assumed as a possible mediator of the ventilatory response to hypoxia by acting on central breathing control in the brainstem. The objective of our study is to determine if the NO pathway is implicated 1) in the increase in normoxic and acute hypoxic ventilation in Epo-TAg^h mice; 2) in ventilatory acclimatisation to hypoxia in both Wild Type and Epo-TAg^h mice.

Methods: For this study, twenty male anemic SV-40 T antigen (Epo-Tag^h) and twenty four male wild-type (C57Bl6/CBA, WT) mice, 8 weeks-old, were divided into four groups: a) Normoxic Epo-TAg^h (Nx Epo-TAg^h; $n = 10$); b) Normoxic Wild Type (Nx WT; $n = 12$); c) Hypoxic Epo-TAg^h (Hx Epo-TAg^h; $n = 10$) and d) Hypoxic Wild Type (Hx WT; $n = 12$).

At the end of the 2 weeks of exposure to hypoxia (4500 meters) the medulla were removed to measure the concentration of NO metabolites (NOx) by elisa, the mRNA and protein of iNOS and nNOS by real time RT-PCR and western blot.

Results: As previously shown hypoxic ventilatory response as well as ventilatory acclimatisation to hypoxia are enhanced in Epo-TAg^h.

In normoxia, NOx was lower only in plasma (−45%) and unchanged in medulla while iNOS protein was higher in medulla in Epo-TAg^h vs. Wild Type mice. Exposure to chronic hypoxia resulted in a marked increase in NOx in plasma and medulla of Wild Type (+48%, +42% respectively) and Epo-TAg^h mice (+45%, +185%). These changes were accompanied by an increase in iNOS expression in the medulla of hypoxic vs. normoxic Wild Type mice and a similar although insignificant rise in hypoxic vs. normoxic Epo-TAg^h mice.

Conclusion: These results demonstrate that 1) acclimatisation to hypoxia leads to an increase in NO production, even larger in the central nervous system in Epo-TAg^h mice; 3) the increase in NOx, essentially through an increase in iNOS in the medulla, could account for the improvement of hypoxic ventilatory acclimatisation in Wild Type mice and probably in Epo-TAg^h anemic mice.

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Differential involvement of Rho-kinase in the contractile response to cholinergic stimulation of rat airways

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Introduction: The aim of this study was to investigate the role of Rho-kinase in isometric force development in response to cholinergic stimulation in rat airways.

Methods: Isometric contraction was recorded from trachea (T), extrapulmonary (EPB) and intrapulmonary bronchus (IPB) rings from Male Wistar rats (250–300 g). Maximal response to 1 mM ACh was used as reference response, then rings were stimulated by 0.3 μM or 10 μM carbachol (CCh), in the absence (C) and in the presence of the Rho-kinase inhibitor Y27632 (10 μM) administered 15 min prior CCh stimulation. Derivative of force on time showed that the contraction occurred in 2 phases: a fast early response (200 s), and a delayed response (30 min). The fast phase was fitted by Hill equation for determination of fast maximal contraction (fFmax), time to half-fFmax, (Tf50), and Hill coefficient (n). The amplitude of the additional delayed contraction (adFmax) was the difference between the delayed maximal contraction and fFmax. Results are expressed as mean ± SEM. Statistical comparison were done using ANOVA, and considered significant when $P < 0.05$. N = number of rats.

Results: In C experiments (N = 8), in response to 10 μM CCh, fFmax was in T: 77.00 ± 2.62%ACh; in EPB: 92.63 ± 6.24% ACh; in IPB: 113.78 ± 7% ACh. Tf50 was in T: 17.38 ± 1.62 s; in EPB: 17.07 ± 1.51 s; in IPB: 19.67 ± 1.07 s. N was in T: 1.91 ± 0.22; in EPB: 2 .52 ± 0.31; in IPB: 3.30 ± 0.33. In IPB, fFmax and n were significantly higher than in T. In response to 0.3 μM CCh (N = 8), fTmax was lower and Tf50 higher compared to 10 μM CCh stimulation, and there was no additional delayed contraction. FFmax was in T: 35.27 ± 6.50 % ACh; in EPB: 43.18 ± 10.29% ACh; in IPB: 27.57 ± 5.42% ACh. Tf50 was in T: 90.85 ± 13.09 s; in EPB: 53.69 ± 10.10 s; in IPB: 42.25 ± 12.87 s. Incubation with Y27632 did not modify the baseline tension. In response to 10 μM CCh, Y27632 significantly reduced fFmax (T: 46.83 ± 4.36% ACh; EPB: 54.47 ± 3.74% ACh; IPB: 49.30 ± 3.68% ACh), but not Tf50, n, or adFmax, and also abolished the differences between T and BIP. Y27632 abolished almost completely the contractile response to 0.3 μM CCh (T: 2.67 ± 0.80% ACh; EPB: 5.22 ± 2.08% ACh; IPB: 2.65 ± 1.74% ACh; N = 7–8).

Conclusion: Upon airway cholinergic stimulation, Rho-kinase activity is implicated in the fast early phase of the contraction, influencing force amplitude, but not force development velocity. It is not involved in the additional, delayed contraction. The relative importance of Rho-kinase in airway contraction also depends on the intensity of cholinergic stimulation and the position along the airway tree.

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Effect of a gain-of-function mutation of the beta-ENaC gene in adult mouse alveolar fluid balance

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Introduction: Transepithelial sodium (Na^+) and water transport across the alveolar epithelium regulates the volume of fluid in the alveolar space and represents the main mechanism for removal of alveolar edema fluid. The amiloride-sensitive epithelial sodium channel (ENaC) expressed in the apical membrane of alveolar epithelial cells (AEC) is considered as a rate-limiting step for alveolar Na^+ transport. We examined whether a gain-of-function mutation of the beta-ENaC gene would affect transepithelial alveolar Na^+ and water transport and modulate the severity of pulmonary edema in mice.

Methods: Transgenic mice harboring the Liddle R566-stop gain-of-function mutation (beta-ENaC C-terminus deletion leading to decreased ENaC endocytosis and increased cell surface expression) were studied. ENaC subunit expression was assessed by real time RT-PCR and Western blotting in wild type (+/+) or mutated (L/L) mouse AEC. Alveolar fluid clearance (AFC) was measured *in vivo* in an *in situ* lung model under basal and beta2-agonist-stimulated conditions. Wild type and (L/L) mice were finally exposed to acute volume overload by saline infusion (40% body weight within 2 h), and the severity of hydrostatic pulmonary edema was assessed by the bloodless wet/dry (W/D) lung weight ratio.

Results: α- and gamma-ENaC mRNA and protein levels were unchanged in Liddle mice (L/L) as compared with (+/+) littermates. AFC increased 2- and 3-folds in +/L and L/L mice respectively, as compared with +/+, due to increased amiloride-sensitive Na^+ transport. Beta2-agonists failed to stimulate AFC in L/L mice. Acute volume-overload significantly increased the bloodless W/D lung weight ratio in both +/+ and L/L mice as compared with baseline, but the increase was more important in +/+ than in L/L mice (5.20 ± 0.366 vs. 4.36 ± 0.175 in +/+ and L/L mice, respectively; $P = 0.01$).

Conclusion: Liddle mice represent the first transgenic model with constitutive hyperactivity of ENaC in the distal lung. They show increased alveolar Na^+ and water reabsorption at baseline, and develop less severe pulmonary edema when exposed to an experimental model of volume overload, suggesting that increased edema fluid clearance protects from alveolar edema in adult lung.

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Impaired alveolar fluid clearance in isolated perfused human lungs correlates well with elevated levels of RAGE, a marker of alveolar epithelial type I cell injury

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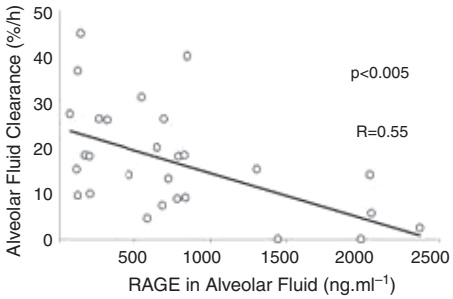
Introduction: Recent studies have established that alveolar epithelial type I cells play an important role in the vectorial transport of excess alveolar fluid from the air spaces of the lung (1) and that the receptor for advanced glycation end-products (RAGE) can be used as a biochemical marker type I alveolar epithelial cell injury (2). The purpose of this study was to test the hypothesis that elevated levels of RAGE would identify those lungs with impaired alveolar epithelial fluid clearance in a novel isolated perfused human lung preparation using lungs rejected for transplantation.



Methods: Human lungs ($n = 30$) were received at 4°C an average of 18 ± 12 h after procurement. The bronchus of a single lung was cannulated and a continuous positive airway pressure of 10 cm H_2O was applied with 100% oxygen. The pulmonary artery was cannulated and perfused at a constant pressure of 12–15 mmHg with Krebs solution containing glucose and 5% albumin. Alveolar fluid clearance was measured with sequential concentrations of protein in the distal air spaces by standard methods. RAGE levels were measured in the alveolar fluid and the perfusate.

Results: The rate of distal airspace fluid clearance (AFC) was inversely correlated with RAGE levels both in alveolar fluid ($P < 0.005$; see Figure) and in the perfusate ($P < 0.05$). A concentration of RAGE above 700 ng.mL^{-1} in the alveolar compartment predicted, with a positive predictive value of 69%, an AFC < 14%, previously defined as impaired clearance (Verghese et al.; *J. Appl. Physiol.*; 1999; 87: 1301–12).

Conclusion: RAGE may be a useful biological marker of alveolar epithelial injury and barrier dysfunction in the human lung, and RAGE may have predictive value for determining the capacity of the alveolar epithelium to resolve alveolar edema in patients with acute lung injury.

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Abstract with drawn

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Antiepileptic drugs in rheumatological pains: recommendations of the CEDR

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Introduction: Neuropathic pain is common in rheumatological practice, although often associated with nociceptive mechanisms. It is caused by lesions or dysfunction of the nervous system, and the usual treatments with analgesics or anti-inflammatory drugs are most often ineffective. Antiepileptic drugs (AEDS) have proven effective in relieving neuropathic pain, mainly diabetic neuropathic pain and post-herpetic neuralgia. AEDs are hardly used by rheumatologists since only recently has attention been drawn to the importance of neuropathic pain in rheumatological conditions. The published trials of AEDs in rheumatological pains are scarce, generally not controlled, with small sample sizes, and many different types of neuropathic conditions are often found in a same trial.

Methods: A systematic review of the published literature was performed by a working group of seven experts from the CEDR (Cercle d'Etude de la Douleur en Rhumatologie) a specific interest group of the French Society of Rheumatology that focuses on rheumatic pain. The search was conducted using electronic databases (Medline and Embase) with no limitations on the type of publication. A series of questions about the prescription of AEDs in rheumatological pains was established and validated by the group. Based on the literature and the clinical experience, each expert responded to the questions and the recommendations were elaborated with the Delphi method.

Results: This work leads to eleven recommendations about the AEDs prescription in rheumatological pains.

Conclusion: These recommendations represent only a guideline for the physicians meanwhile disposing more scientific evidences.

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Compliance with prescribed medication in children after emergency department (ED) visit

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Introduction: Many studies have assessed compliance in adults, especially in chronic diseases, and parameters that influence compliance for these patients. For children, situation is very different as drugs are administered to children by parents. However, noncompliance with pediatric drug therapies seems to be also a common and important problem with studies in children suggesting an overall drug noncompliance rate of approximately 50% for treatment of chronic disease. There is little information about drugs prescribed to children in acute diseases especially in emergency department. For emergency department (ED) patients, compliance with drug therapy is essential as many ED patients do not have short term follow-up evaluation after un ED visit and non compliance could be a cause of subsequent hospital admissions. Few studies have examined the rate of compliance in the primary care or medical clinic setting.

The objective of this prospective study was to determine the rate of compliance with prescriptions from a pediatric emergency department and to assess factors associated with noncompliance.

Methods: Pediatric patients of all ages (0–16 years old) discharged from the pediatric emergency department with at least one oral drug prescription were included. A telephone interview questionnaire was used to determine whether the child had received the treatment according to the prescription and if not, the reason(s) for not doing so. Compliance was assessed using 3 items: the frequency of drug administration, length of treatment and administering method.

Results: One hundred five telephone interviews were exploited. Children were 60 boys (57%) and 45 girls (43%). The age of these 105 children ranged from 0.2 to 12 years (3.5 ± 2.9 years), with 51 (49%) younger or equal than 2 years old. The most common diagnosis was asthma (n = 32, 31%) and pulmonary infection (n = 27, 26%). Complete compliance (respect of the 3 items) with having the prescription was 36.2% (38/105). Some factors were significantly associated with noncompliance ($P < 0.05$) by univariable logistic regression: length of treatment; multiple prescriptions; frequency of doses; sex male.

Conclusion: Prescription of multiple medications, the dosing regimen and the length of therapy play a significant role. If doctors have the opportunity to choose the most acceptable regimen of a medication for their patients, they should take it.

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The therapeutic guidelines database, an original new tool

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Introduction: Physicians are expected to prescribe according to current scientific data and evidence-based medicine, as embodied by official treatment guidelines. In order to apply these, physicians should be aware of ways to access them, and smoothly integrate their content into their daily practice. Our objectives were to provide validated summaries of treatment guidelines, to facilitate access to original documents, and to keep physicians informed on updates and related news.

Methods: For one hundred most frequently encountered diseases, expert writers (GPs and specialists) were chosen to produce a summary of current validated treatment guidelines. The outline and content of these summaries were harmonized in order to increase user-friendliness. Tree-shaped decision algorithms constitute the core of each summary. They are completed with disease and diagnosis information, specific clinical situations, patient information as well as drug and non drug treatment information (all available medicinal products are listed). Whenever possible, these summaries are weighed with grades of recommendation corresponding to scientific levels of evidence, as defined by the HAS (high autority of health).

Results: The therapeutic guidelines database is available through:

- a website which proposes therapeutic guidelines summaries and related information regarding diseases most frequently encountered by GPs. A search engine has been integrated, which allows physicians to locate French and international treatment guidelines. This engine transforms queries expressed in usual French medical terms into MeSH (medical subjects headings) - compatible queries in order to search French and international databases efficaciously. We created a watch team to update the database and inform physicians about the latest available guidelines as well as other recent guideline-related news.
- a pocket book with one hundred treatment strategies based on guideline summaries.

Conclusion: The therapeutic guidelines database is the first one for French-speaking healthcare professionals. It allows access to validated therapeutic guidelines (summaries, full-text guidelines, as well as related news). The next step is to measure the effects of this new tool on GPs daily practice.

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Abstract withdrawn

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Key role of bone marrow estrogen receptor alpha and FGF2 on the effect of estradiol on reendothelialization

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Introduction: 17ß-Estradiol (E2) accelerates reendothelialization, but the cellular and molecular events are poorly defined, mainly due to the lack of appropriate models and tools to visualize this thin cell monolayer. The main aims of this study were, first, to compare the reendothelialization in endovascular injury and perivascular electric injury and second, to determine the respective role of medullary and extramedullary cells.

Methods: Confocal 'en face' immunohistochemical analysis revealed striking differences between E2 and placebo treated mice. E2 increases the BrdU incorporation, endothelial NO synthase and decreases PECAM staining in the reendothelialized area as well as in a 'committed zone' immediately adjacent to the injured area. The reendothelialized area was significantly smaller in non-treated mice, and interestingly, the committed zone was completely absent in these mice. This E2-dependent effect on the activation of the committed zone was similar in both injury models. Thus, SMC do not appear to play a major role in reendothelialization.

Results: These two E2 effects were abolished in estrogen receptor alpha (ERα-/-) mice, as well as in WT mice grafted with ERα-/- bone marrow (BM), whereas it was preserved in mice grafted WT BM, demonstrating an essential role of medullary ERα. As previously demonstrated, E2 increased both the velocity of reendothelialisation and the number of circulating EPCs in ovariectomized wild-type mice. The implication of FGF2 in this process revealed that the E2 effect on both parameters was abolished in FGF2-deficient mice (*Fgf2*-/-), demonstrating that FGF2 is absolutely required for these E2 effects. To test the implication of medullary and extramedullary FGF2, we grafted *Fgf2*-/- bone marrow to *Fgf2* + /+ mice and

observed that the effect of E2 on both reendothelialization and EPC levels was abolished, demonstrating that only BM-derived, but not extramedullary FGF2 is required for both effects.
Finally, WT mice were grafted with GFP expressing BM, allowing the visualization of BM-derived cells. These experiments revealed that E2 increased the number, but not the density, of intimal GFP-expressing cells 3 days post-injury.
Conclusion: In conclusion, the acceleration of reendothelialization and engagement of the 'committed zone' by E2 are two closely related processes, which are both mediated by medullary ERα and FGF2.

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Relationships between endothelial dysfunction and coronary artery stenosis in asymptomatic type 2 diabetic patients
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Introduction: Silent myocardial ischemia (SMI) when associated with coronary artery stenosis or endothelial dysfunction is predictive of cardiovascular events in type 2 diabetic patients. The aims of the present study were to examine the relationships between peripheral and coronary endothelial functions (PEF and CEF), left ventricular (LV) function and the presence of significant coronary artery stenosis in asymptomatic type 2 diabetic patients.
Methods: Fifty type 2 diabetic asymptomatic patients (36 men; aged 61 ± 7 years, mean duration of diabetes 14 ± 8 years) with a normal resting ECG were included. They were tested for SMI defined as an abnormal ergometric stress test, dipyridamole myocardial scintigraphy or dobutamine stress echocardiography. A coronarography was performed when SMI was found. PEF was assessed by post-occlusive hyperemia of the brachial artery (n = 50) and CEF by coronary trans-thoracic Doppler during a cold-pressor test (CPT) (n = 25).
Results: Patients were classified in three groups: group 1: no SMI (n = 26); group 2: SMI, no significant coronary artery stenosis (n = 17) and group 3: SMI and significant coronary artery stenosis (n = 6). Although hyperemia induced a similar increase in mean blood flow velocity in the three groups, the variations in brachial artery diameter (diameter at one min./basal diameter) were different between group 3 (0.96 ± .05) and group 1 (1.01 ± .04, $P < 0.01$) and 2 (1.01 ± .04 $P < 0.01$). There was a correlation between the changes in brachial artery diameter and the LV relaxation index E/A (r = 0.32; $P < 0.05$), but not with the left ventricular mass which was similar in the three groups of patients. CPT induced a similar increase in mean coronary blood flow velocity and velocity time integral in the three groups of patients. No correlation was found between PEF and CEF.
Conclusion: Peripheral endothelial function is altered in asymptomatic type 2 diabetes patients with significant coronary artery stenosis and endothelial dysfunction is associated with an altered LV relaxation.

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Red wine polyphenols, Provinols™, improve endothelial function via an increase of nitric oxide production and a reduced oxidative stress in Zucker fatty rats Fa/Fa
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Introduction: Obesity is associated with numerous complications including significantly increased risks of diabetes and cardiovascular diseases. Epidemiological studies report an inverse association between dietary flavonoid consumption and mortality from cardiovascular diseases. The aim of this work was to study the vascular effects of dietary supplementation of red wine polyphenols extract, Provinols™, in an experimental model of obesity, the Zucker fatty rats (ZF).
Methods: Rats (male, 6 week old) received normal diet (n = 5) or supplemented with Provinols™ (20 mg/kg/day, n = 6) for 8 weeks. Then, vascular reactivity was assessed and tissular content of nitric oxide (NO) and superoxide anion (O_2^-) production was measured by electronic paramagnetic resonance. Statistical analysis were performed by one way analysis of variance (ANOVA), and Mann-Whitney U tests or tow way ANOVA for repeated measures and subsequent Bonferroni post hoc test. $P < 0.05$ was considered to be statistically significant.
Results: Treatment with Provinols™ significantly reduced the change in body weight by 11%. Systolic blood pressure was not different among the groups and was neither affected by Provinols™. In aorta, Provinols™ improved endothelium-dependent relaxation to acetylcholine (Ach; EC50; 138 nM vs. 65 nM) without affecting that of the NO donor, sodium nitroprusside. Provinols™ did not modify contraction induced by phenylephrine (Phe). The NO-synthase inhibitor, N^{ω}-Nitro-L-arginine methyl ester (L-NAME, 100 μM) increased contraction to Phe to the same extent in vessels from control or treated groups (EC50; 14.8 nM and 15.1, respectively). The non-selective cyclooxygenase (COX) inhibitor, indomethacin ($3x10^{-6}$ M), and the COX-2 selective inhibitor, NS398 (10^{-6} M), significantly reduced the response to Phe in the aorta from both groups [(71% vs.. 57% in Provinols™, for indomethacin) and (51% vs. 48% in Provinols™, for NS398)]. However, the contraction to the agonist was greater in Provinols™ in the presence of indomethacin. In small mesenteric arteries, Provinols™ did not affect both the endothelium-dependent relaxation to Ach and the contraction to Phe. Provinols™ reduced O_2^- content in aorta, carotid and small mesenteric arteries (2.65, 3.2 and 2.2 fold, respectively), but enhanced their NO content (1.4, 3.4 and 2.2 fold, respectively). These effects were associated with reduced NF-κB and Nox-1 stainings in the aorta.
Conclusion: Provinols™ increase NO production, reducing both oxidative stress and inflammatory process both in conductance and resistance arteries from ZF rats. This effect is associated with improved endothelial function and reduction of the participation of vasoconstrictor metabolites from COX within the aorta. Altogether, these results point out beneficial effects of plant-derived polyphenols on endothelial function and vascular reactivity in obese rats.

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The protective effect of statins against monocrotaline-induced pulmonary hypertension might not be truly a class effect
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Introduction: Pulmonary artery hypertension (PAH) is an uncommon, yet devastating, syndrome with a complex pathobiology. Pravastatin (PS), a natural hydrophilic statin, prevents monocrotaline-induced PAH in the rat[1].The objective of this study is to compare a lipophilic synthetic statin, atorvastatin (AS), to PS, a natural hydrophilic statin.
Methods: PS or AS (both at 10 mg/kg/day) or vehicle were given orally for 28 days to Wistar male rats injected or not with monocrotaline (MC, 60 mg/kg intraperitonealy). Right ventricular pressure (RVP) and right ventricular hypertrophy (RVH = right ventricle/left ventricle plus septum ratio) were assessed as results of PAH. Histological examination as well as immunohistochemistry and western blot experiments for endothelial NO synthase (eNOS) and cleaved caspases-3 expression were performed on the lung and pulmonary arteries. Endothelium-dependent (Ach) and independent (Sodium Nitroprusside, SNP) vasodilatations of main pulmonary arteries (PA) were studied.
Results: As previously reported[1], at 4 weeks, MC-injected rats developed severe PAH, with an increase in RVP and RVH associated with a decrease in Ach- or SNP-induced (PA) dilation observed *in vitro* as well as a decrease of endothelial nitric oxide synthase (eNOS) expression and an increase of endothelial cell apoptosis and of PA medial thickness. PS (P = 0.02) but not AS (P = 0.30) significantly limited the development of PAH (RVP in mmHg: 30 ± 3, 36 ± 4 vs. 45 ± 4 and 14 ± 1 for MC + PS, MC + AS, MC and control groups respectively). Both statins significantly reduced MC-induced RVH (RV/LV + S, in mg/g: 0.46 ± 0.04, 0.39 ± 0.03, 0.62 ± 0.05 and 0.29 ± 0.01 for MC + PS MC + AS, MC and control groups respectively, $P < 0.05$), and reduced MC-induced thickening (61 ± 6 μm, 82 ± 5 μm, 154 ± 4 μm and 59 ± 2 μm for MC + PS, MC + AS, MC and control groups, respectively, P = 0.01) of small intrapulmonary arteries medial wall, with MC + AS still being different from control group. PS, but not AS, partially restored Ach-induced pulmonary artery vasodilation in MC-rats (Emax = 65 ± 5%, 49 ± 6%, 46 ± 3% and 76 ± 4% for MC + PS, MC + AS, MC and control group respectively, $P < 0.05$ for MC + PS vs. Other groups). Both statins prevented apoptosis and restored eNOS expression of pulmonary artery endothelial cells, as well as in the whole lung, with PS being more efficient than AS.
Conclusion: Despite its effects on eNOS expression, apoptosis and medial wall thickening, AS was unable to significantly reduce PAH and to restore endothelium-dependant relaxation, suggesting intermolecular differences between the two HMG-CoA reductase inhibitors in the protection against MC-induced hypertension.

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Lack of endothelial dysfunction in Buerger disease, influence of endothelin ETA ETB receptor antagonism
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Introduction: Buerger disease is a non inflammatory, non atherosclerotic arteritis involving medium size arteries and leading to early amputation. Its pathogenesis is unknown but may involve endothelial dysfunction and endothelin hyperactivation. Our aim was to compare the acute flow-dependent vasodilatation (FDV) and endothelium-independent vasodilatation (EIV) of the brachial artery (BA) and aortic stiffness in 10 patients with an acute-phase Buerger disease and 10 age- and sex-matched non-smokers healthy subjects.
Methods: BA diameter and shear stress were recorded by high definition echotracking. FDV was estimated by the slope of diameter-shear stress relationship during hand warming test (from 28°c to 44°C) and after GTN (150 μg), before and after administration of tezosentan (Tez), an ET-A-ET-B receptor antagonist (double blind cross-over acute administration). Aortic stiffness was measured with carotid-femoral pulse wave velocity (CF-PWV).
Results: Buerger's patients had an enhanced flow-dependent response to the increase in shear stress due to hand warming by comparison with controls as shown by the higher slope of diameter-shear stress relationship. CF-PWV was increased in Buerger by comparison to controls (10%, $P < 0.05$). Blood pressure decreased significantly with Tez. Tez had no influence on endothelial function, but improved CF-PWV independently of blood pressure reduction (−8%, $P < 0.05$).

Median (IQR)		Control	*P*-value
FDV:ΔBA diameter (%)	6.8 (0.1;8.1)	3.7 (1.1;6.5)	ns
FDV:Δshear stress (%)	70 (32;100)	93 (57;125)	ns
EIV:ΔBA diameter (%)	30 (28;33)	17 (13;22)	ns
EIV:Δshear stress (%)	98 (40;137)	117 (76;251)	ns
Slope diameter-stress	0.89 (0.55;1.24)	0.28 (−0.01;0.58)	0.025

Conclusion: Acute flow-mediated changes in brachial artery diameter during hand hyperhemia and EIV to GTN were not impaired in patients compared to control, by contrast to what has been repeatedly suggested in the literature. Endothelin system seems to be involved in aortic stiffnening, not in endothelial hyperresponse.

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Impact of safety alerts on measures of disproportionality in spontaneous reporting databases: the notoriety bias
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Introduction: Disproportionality analysis of spontaneous reporting is increasingly used routinely, but it may be influenced by notoriety due to safety alerts which has yet to be explored. We wanted to study the consequences of safety alerts on reporting disproportionality.

Methods: Within the French Pharmacovigilance database, disproportionality of reporting was tested before and after four alerts: valvulopathies with pergolide, tuberculosis with infliximab, strokes with atypical antipsychotics and rhabdomyolysis with statins (after cerivastatin withdrawal), using the case non-case approach.
Results: No valvulopathy was reported with pergolide before the alert but 63 cases were reported after (Reporting Odds Ratio ROR = 9369; 95% CI: 4338–20 237), of which five had occurred before the alert; 25 reports mentioned rhabdomyolysis with statins (not including cerivastatin) before the alert (ROR = 5.8; 95% CI: 3.8–9.0), and 63 after (ROR = 9.4; 95% CI: 7.0–12.6). Approximately 280 reports concerning cerivastatin were notified after its withdrawal. There were two reports of tuberculosis with infliximab before the alert (ROR = 1529; 95% CI: 134–17 458) and 7 after (ROR = 434; 95% CI: 111–1703). One report mentioned stroke with atypical antipsychotics before the alert (ROR = 0.09; 95% CI: 0.01–0.64) and 16 after (ROR = 1.13; 95% CI: 0.69–1.87). After excluding events involving antiplatelets and anticoagulant agents, ROR were 0.14 (95% CI: 0.02–1.03) before the alert and 2.02 (95% CI: 1.21–3.35) after.
Conclusion: Disproportionality in spontaneous reporting databases increases after a safety alert, because of increased reporting of the event of interest, including events occurring before the alert. This may overflow to increased reporting of the event with other drugs e.g., three tuberculoses with other drugs after the alert vs. One before, or reporting of rhabdomyolysis with statins other than cerivastatin. Reporting disproportionality should not be tested once an association between a drug and an event has been identified, and only with caution when an alert has already been raised with similar products.

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Meningococcal serogroup B vaccine (MenBvac®): pharmacovigilance follow-up of the vaccination campaign in the department of Seine-Maritime (Upper-Normandy)

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Introduction: Since 2003, an increase of the incidence of invasive meningococcal infections, particularly of serogroup B (14: P1–7, 16), was noticed in Seine-Maritime (Upper-Normandy). The *French Technical Committee on Immunisation* has proposed a vaccination campaign targeting children and young adults from 1 up to 19 years old, living or studying in Seine-Maritime, with the use of a Norwegian meningococcal OMV vaccine MenBvac® produced by the NIPH (*National Institute of Public Health*). As MenBvac® does not have MA, French Health Ministry assumes the responsibility in term of dispensation and administration for the vaccine in accordance with the *L31-10* article for the code of Public Health. Because of few safety data available for the vaccine of young children, an 'active' pharmacovigilance follow-up was set up by the AFSSAPS. The objective of this study was to evaluate the nature, seriousness and incidence of adverse effects (AE) occuring within the 15 days following immunization.
Methods: The regional centre of pharmacovigilance (CRPV) of Rouen in charge of this follow-up collected the immediate and the late AE by using a specific reporting form, filled out by the vaccine administrators and/or the vaccine recipients/or parents. The campaign began on June 12, 2006, targeting children from 1 up to 5 years old, living in a limited area of the department (Dieppe) by using a total of 9000 vaccine doses available. A total of 2891 and 2869 children (70% of the target population) received a 1st (D1) and a 2nd dose (D2) six weeks later, respectively.
Results: Following D1, a total of 2662 forms (92%) were sent back to the CRPV. 1240 children (47%) presented at least an AE; 91 of them (7.3%) consulted a general practitioner (GP) and a child (0.1%) presented a serious AE, linked to a probable idiopathic thrombocytopenic purpura. Concerning D2, the CRPV received a total of 1609 forms (56%): 527 children presented at least an AE, i.e. 33%; 25 of them (4.7%) referred to a GP and a child (0.2%) was hospitalized for a feverish vascular purpura, occurring in a context of viral infection, for which a meningococcal infection was ruled out. Local AE (pain, redness and tumefaction at the injection site) for less than 15% and fever for less than 20% were reported for the vaccine recipients. Other frequent, moderate and transitory reactions were the followings: digestive disorders (vomiting, diarrhoea, nausea, abdominal pain), headache, agitation, nervousness, irritability, sleep disorders (insomnia, nightmares), benign cutaneous eruptions, myalgia and asthenia.
Conclusion: This current active pharmacovigilance follow-up has not identified any safety signal. The most frequent case-reports concern benign and listed AE, mainly fever and local effects, previously reported during clinical trials carried out by the NIPH with MenBvac® (1) administered in the same age group.

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Spontaneous reporting of adverse drug reactions by general practitioners or psychiatrists: a comparative survey from a pharmacovigilance database

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Introduction: Spontaneous reporting (SR) is one of the most important early warning system of adverse drug reactions (ADRs) detection for marketed drugs. Under-reporting of ADRs is significant and can lead to underestimation or late detection of safety signals. Knowledge of healthcare professionals habits for SR, could be of interest to understand mechanisms of under-reporting. The aim of the study was to compare general practitioners (GPs) and psychiatrists for demographic profile, practices, knowledge and habits in pharmacovigilance.
Methods: The study design is a descriptive, comparative and retrospective study on a sample of GPs and psychiatrists. The sample was randomly obtained from the French Lundbeck pharmacovigilance database, among practitioners who reported adverse drug reaction, from the year 2004 to 15 September 2006, associated to citalopram or escitalopram. Both drugs are Lundbeck's SSRI antidepressant. A self-administered, anonymous and validated questionnaire was sent to 182 GPs and 182 psychiatrists, in September 2006. Four weeks later, a reminder was sent.
Results: The number of completed questionnaires returned was 159 (44%), of which 82 (45%) were from GPs and 77 (42%) from psychiatrists. Compared to GPs, psychiatrists were older (more psychiatrists are 50-years-old or more), more often located in large cities (33% vs. 4%) as expected according to existing national data. No difference was found regarding educational level in pharmacovigilance (27% vs. 31% specified to have never had training in pharmacovigilance). More than 70% of the responders stated to have reported between 1 and 5 ADRs during the last twelve months, which were mainly reported to the manufacturer and less to a regional pharmacovigilance center. Whereas the psychiatrists are less often visited by sales representatives (between 0 and 5 visits per month) compared to GPs (16 or more), they report more often an ADR via a sales representative (32% vs. 26%). The most important factors leading to reporting seem to be: the need for information regarding the ADR or the seriousness of the reaction. Some effective measures proposed to improve reporting could be the availability of a toll-free phone number or a pre-completed questionnaire.
Conclusion: No major difference appears between GPs and psychiatrists regarding demographics. Differences were observed mainly concerning areas of residence and practices, as expected. A lack of pharmacovigilance training is stressed by both GPs and psychiatrists. In that study, the role of the sales representatives in the ADR reporting seems to be not negligible especially for psychiatrists.

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Ischemic and/or thromboembolic events during anti-TNF therapy: results of a 6-year French national survey

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Introduction: As several cases of ischemic and/or thromboembolic events (ITE) have been recently reported during anti-TNF therapy, the aim of this work was to study retrospectively, over a 6-year period, the main characteristics of spontaneously notified TNFα antagonists-related ITEs in the French adverse drug reaction (ADR) reporting system database.
Methods: Spontaneously notified cases of ITE during anti-TNF therapy (infliximab, etanercept and adalimumab) were collected by the French pharmacovigilance network (31 Regional Centers) between January 2000 and March 2006. Patients' characteristics (sex, age at ITE onset, initial disease and treatment duration) were recorded. ITE were then classified according to their arterial or venous origin. Risk factors (RF) assessment for ITE was then made according to consensual criteria. French imputation method was used to assess the causality between ITE and TNFα inhibitor-related ADR.
Results: During this period, 77 cases of ITE (each one corresponding to 1 patient) were identified among 1521 notifications of TNF-related ADRs (5.1%). No significant trend was found between the 3 TNF-α inhibitors. Initial disease was mainly RA (n = 54) and, to a lesser extent, spondylarthropathies (n = 13), Crohn's disease (n = 3), ulcerative colitis (n = 3), pustular psoriasis (n = 1), cicatricial pemphigoid (n = 1) and was not determined in 2 patients. In this population, 36 arterial and 41 venous ITE were identified. Analysis showed that 44.4% of patients showing arterial ITE had 2 or more RF whereas a main proportion (90%) of patients presenting venous ITE had no or only one RF. Causality was dubious in 74 ITEs and possible in 3 patients. ITE was considered as serious in 72 patients and non-serious in 5 cases. ITE outcome was: lethal for 5 patients, with after-effect for 7, with partial recovery for 20, favourable for 38, and unknown for 7 patients. Mean duration of TNF antagonist therapy at ITE onset was 10 months. Among them, 13 ITE occurred during the first month, 2 happening the day after treatment initiation. Etanercept appeared to be significantly associated with a shorter delay of onset, particularly for venous ITE (2.6 months). Except one, all patients received concomitant therapy: systemic corticosteroids (CS, n = 53), methotrexate (MTX, n = 31) and COX-2 inhibitors (n = 6). Additionally, 25 patients were co-treated by both CS and MTX. Only 23 patients were investigated for autoantibody panel: antinuclear positivity was present in 9 patients and 4 patients were positive for antiphospholipid auto-antibodies.
Conclusion: This descriptive 6-year nationwide survey suggests that venous ITEs could be favoured by anti-TNF therapy as they occurred in patients with no or few RF for venous thrombosis (auto-immunity ?). In contrast, the relationship between arterial ITE and TNFα blockers appears weaker since (1) most of these patients often exerted several independent arterial RF and (2) that the initial disease, via systemic inflammation, could often be considered as a confounding factor.

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PharmacoMIP: first results of a pilot study of a regional hospital-based pharmacovigilance network

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Introduction: Under-reporting of adverse drug reactions (ADRs) remains a limit of spontaneous reporting method in pharmacovigilance. About 70% of ADRs reported to Center of Pharmacovigilance of Toulouse comes from toulouse university hospitals (TUH). This fact could be explained by our method of collect of ADRs, i.e. regular visits of residents from the pharmacovigilance center to different departments of TUH. Then, we hypothetized that a same design performed in public and private hospitals could improve the rate of ADRs' reports out of TUH. In 2005, we suggested to RAH (Regional Agency of Hospitalisation), the recruitment of one clinical research assistant (CRA) for regular visits to regional hospitals or clinics (out of TUH) to collect ADRs. This study was supported by RAH and DRASS (Direction Regionale des affaires Sanitaires et Sociales) of Midi-Pyrénées.
Methods: The study was set up in April 2006 and two departments were selected for the pilot study: Haute-Garonne and Gers with respectively 41 and 14 public or private hospitals (total = 55). A letter explaining the objective and the method of our study was mailed followed by our visit in all hospitals in order to present the project and discuss about the method adapted to each one from May to November 2006. The CRA began the regular visit in September 2006.
Results: During 3.5 months (until 15 December 2006), 180 ADRs were collected in 24 hospitals. About 46% of ADRs were 'serious' (leading to hospitalization or prolongation of hospitalization and one case of death suspected to psychotrops). All cases were analyzed and registered in the national pharmacovigilance database. Compared to the same period in 2005, the rate of reporting of these hospitals increased by two fold. The characteristics of ADRs will be discussed. Moreover, informations about drugs were required by practitioners in 20 cases.
Conclusion: The first results of our pilot study clearly show that the regular visit of a member from the regional center of pharmacovigilance decreases the rate of under-reporting of ADRs in hospitals and contributes to the rational drug use by providing independent data about drugs. Better results are expected for 2007 after the set up of this system in all hospitals. Other CRAs are also necessary in order to extend this study in the whole Midi-Pyrénées area.

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Adverse drug reactions in patients older than 70 years during two heat waves in France (2003 and 2006)

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Introduction: Exceptional heat waves have occurred in France during summers 2003 and 2006. Among the different risk factors of hosph-mortality during these periods, drugs were reported to be one of the possible causes. A precedent study underlined the role of the 2003 heat wave in the occurrence of serious adverse drug reactions (ADRs) in elderly (1). The objective of this work was to compare ADRs in patients older than 70 years reported to the French Network of Pharmacovigilance during the 2006 summer heat wave with those occurred during 2003.

Methods: All 'serious' ADRs occurred in patients older than 70 years between 1st July and 31st August 2006, reported to the French Network of Pharmacovigilance Centres and recorded in the French PharmacoVigilance Database were analysed with respect to age, gender, type of ADR, drug involved, evolution as well as imputability of heat wave (IHW). The last item was defined after full revision of the whole reports. Each report was reviewed by two of us to determine the role of heat in ADR using a scale ranging from *unlikely* (IHW0), *possible* (IHW1), *plausible* (IHW2) and *likely* (IHW3). Data of 2006 were compared to the data observed in 2003.

Results: The total number of 'serious' ADRs registred in the French PharmacoVigilance Database in patients older than 70 years was similar in 2003 (n = 304) and 2006 (n = 353). Seventy-two patients were concerned (29 'IHW1', 29 'IHW2' and 14 'IHW3'), with a maximal peak between the 10th and the 29th July. The most frequently ADRs were metabolic (dehydratation, hydroelectrolytic disturbances), neuropsychiatric (confusion, sleepness), renal (acute renal insufficiency), general with hyperthermia. Drugs more frequently involved were diuretics, angiotensin receptor antagonists (sartans), angiotensin converting enzyme inhibitors, antiparkinsonians, anti infectious, antidepressant (mainly serotonin reuptake inhibitors), neuroleptics, digoxin and oral hypoglycemics.

Two main differences were found with 2003 heat wave: less antidepressant and hosphorgical drugs were concerned and more antiparkinsonian, antiepileptic, hypoglycaemic and anti infectious drugs were involved for a similar total number of reported ADRs linked to heat wave (68 in 2003, 72 in 2006).

Conclusion: The main characteristics of serious ADRs occurred in patients older than 70 years during heat wave were similar in 2003 and 2006. Diuretics were most frequently involved, whatever the year of study. However, different pharmacological classes were concerned in 2003 and 2006. The decrease of antidepressant and hosphorgical drugs could be explained by a better caring after lessons from 2003. The increase of antiparkinsonian, antiepileptic, hypoglycaemic and anti infectious drugs may identify patients at risk, with other diseases.

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'Contrat de bon usage' and pharmacovigilance

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Introduction: A French decree about the good medical practice concerning drug prescription, called the '*contrat de bon usage*', has been published in 2005. This '*contrat de bon usage*' is signed by the director of regional agency of hospitalisation and the legal representative of all public and private hospitals of the region. It is established for 3–5 years. The issue of this contract is to determine the actions developed by each hospital to improve and secure the medicinal product circuit from prescription to recipient. It aims to assess health professional practice in their implementation for achieving these objectives. In this paper, we are wondering about the contribution of pharmacovigilance (PV) activities to help hospitals to answer the purpose of this contract.

Methods: We postulated that the good use of PV tools like signal detection by notification is an essential step to risk's management which is highlighted in the 'contrat de bon usage'. Through the annual assessment of the regional pharmacovigilance centre (CRPV) we searched indicators showing knowledge and a good application of PV rules.

Results: Some of PV data collecting by the CRPV are gathered in 3 topics reflecting the health public politics, the culture of risk and the reactivity of the system in the matter of drug use:

The indicators can be given hospital by hospital. A regional public health approach can be proposed in respect of geographical sectors and hospitalisation bed numbers.

Health public politics of the hospital	Risk's culture of health professionals	Reactivity of the PV system
1. Identification of a local PV correspondent 2. Quantitative values of the PV notification like adverse drug reaction, medicinal errors and quality product defects	1. Qualitative values of PV notification as serious, unexpected and preventable ADR 2. Number and classification of questions to the CRPV	1. Actions of prevention after a local signal 2. Contribution to the national prospective follow-up of a molecule or a pharmacologic class 3. Respect of the legal delay

Conclusion: In the concept of '*contrat de bon usage*' and pharmacovigilance, the CRPV collects the PV data for public and private hospitals. Then, it becomes a regional observatory of drug iatrogenic disease and a data provider on the quality of prescription for each hospital and for the regional administrative authority.

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Use of laxatives without medical prescription: assessment of stimulant laxative misuse or abuse

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Introduction: Stimulant laxatives can cause serious medical disorders in long term users such as hypokalemia and pseudomelanosis coli. The aim of this study was to describe a population of over-the-counter laxative users and to evaluate misuse or abuse of stimulant laxatives.

Methods: A prospective study was performed among a random sample of 151 pharmacies in the Aquitaine, region of France, to identify all sales of laxatives without medical prescription during a 2-week period (19th of June – 2nd of July, 2006). Data on characteristics of users (age, sex, morphology and type of customer), laxatives use (drug name, number of units, daily dose, duration, frequency and reason of use) and other medicines were collected in a structured questionnaire. The users of stimulant laxatives were compared to the users of other laxatives. Participating pharmacists were also asked to give their opinion on laxative misuse or abuse.

Results: Twenty nine pharmacies participated (participation rate 19.2%); 137 users were included: 86.1% were female and 64.2% were known customers of the pharmacy. The median age was 60 years. Laxatives were used for more than one year by 59.9% of the users and daily by 35.8%. The reasons for laxative use were chronic constipation (44.5%), occasional constipation (26.3%), 'It is the only thing that works' (11.7%), weight loss (8.0%), due to a current drug treatment (3.6%), other reasons (3.6%). More than one laxative brand was purchased by 18.2% of the users. Stimulant laxatives were the most commonly used (62.0%) followed by hyperosmotic agents (18.2%), lubricant agents (14.6%), suppositories or enema (13.1%) and bulking agents (10.2%). The users of stimulant laxatives compared to the users of other laxatives was more often female (P = 0.0038) and evoqued more frequently 'It is the only thing that works' (P = 0.0047). Among the users of stimulant laxatives, chronic users (57.6%) and those who used laxatives for weight loss (8.2%) could be considered as potential misusers or abusers of stimulant laxatives. Overall, on these two criteria, 60.0% of the users of stimulant laxatives were estimated to be potential misusers or abusers. Furthermore, among pharmacists, 55.2% considered laxative misuse or abuse as a problem and 13.8% thought that it was an increasing phenomenon.

Conclusion: Misuse or abuse of stimulant laxatives is far from hosphorg and concerns mostly an elderly population. This could have serious medical consequences. Guidelines on constipation treatment would be useful as laxatives have only a place in acute cases.

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An indirect PK-PD model to assess interaction between acenocoumarol and amoxicillin + clavulanic acid

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Introduction: Acenocoumarol, a vitamin K antagonist, is widely used in Europe. Because of its widespread and long term use, acenocoumarol is frequently associated with other drugs. Amoxicillin plus clavulanic acid is a largely prescribed antibiotic combination. Thanks to clinical observations giving evidence of pharmacodynamic variations of acenocoumarol during concomitant administration of amoxicillin plus clavulanic acid, we decided to investigate the influence of amoxicillin plus clavulanic acid drug interaction on PD marker using a population pharmacokinetic–pharmacodynamic (PK-PD) model.

Methods: A total of eight healthy volunteers were enrolled. Each subject received at day 1 a single dose of 8 mg of acenocoumarol. Then 1 g of amoxicillin +250 mg of clavulanic acid was given from day 3 to day 9. On day 8, each subject received a single dose of 8 mg of acenocoumarol concomitantly to the antibiotic drug. Eleven blood samples were taken during 48 hours following each acenocoumarol administration yielding a total of 176 blood samples. Acenocoumarol plasmatic concentrations and prothrombin time were measured at each blood sample. We first identified the PK structural model by pooling this trial with individual data from other PK acenocoumarol trials (1). Indirect response model was used to fit PD data (2). Models were built using a non-linear mixed effect modelling approach with NONMEM software. Covariates were tested on PK and PD parameters including antibiotic treatment.

Results: PK data of acenocoumarol was fitted by a two compartments first order input model with log normal inter-individual variability. Weight and antibiotic treatment were found to significantly improve the fit of PK data (based on the objective function), with a 15% decrease in the acenocoumarol clearance in case of antibiotic association ($P < 0.05$). An indirect response model was successfully applied to PK-PD data of acenocoumarol. No covariate and especially the antibiotic treatment effect had significant influence on prothrombin time.

Conclusion: Despite some case reports of clinical suspicions, amoxicillin plus clavulanic has no effect on the pharmacodynamic activity of acenocoumarol, as assessed by prothrombin ratio.

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Nelfinavir-M8 pharmacokinetic modeling of placental transfer, a population study

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Introduction: A population pharmacokinetic model was developed in order to describe the transfer of nelfinavir and its active metabolite M8 from maternal to cord plasma and amniotic fluid.

Methods: Individual characteristics that may influence nelfinavir-M8 concentrations were investigated. This study included 75 women on delivery day for whom maternal, umbilical plasma and amniotic fluid samples were collected. To these, 53 pregnant, 61 non pregnant and 7 consecutively pregnant and non pregnant women were added to the database. Data were analysed with NONMEM.

Results: Nelfinavir and M8 concentrations in maternal plasma, umbilical plasma and amniotic fluid were described as 6 connected compartments. Mean population estimates (pharmacokinetic and inter-subject variabilities in %) were: absorption rate 0.67 h^{-1}, lag time 0.87 h, apparent nelfinavir elimination clearance and volume of distribution: 39.5 L/h (53%), 557 L for non pregnant + pregnant women and 115 L/h (132%) and 1626 L on the day of delivery, M8 formation clearance 0.77 L/h and M8 elimination rate constant 3.41 h^{-1} (74%). For nelfinavir and M8 respectively, the mother-to-cord parameters were 0.058 L/h (34%), and 0.35 h^{-1} (76%), the cord-to-amniotic fluid rate constants were 0,23 and 0.59 h^{-1}, and elimination rates from amniotic fluid were 0.36 and 0.49 h^{-1}. Nelfinavir fetus-to-maternal concentration ratio was approximately 25% for maternal concentrations between 0.1 and 2.5 mg/L, in mothers between the 31st and 41st week of gestation.

Conclusion: The weak nelfinavir placental transfer should not contribute to protect the fetus from vertical HIV-1 transmission.

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APOMYGRE: a multicenter trial which validates mycophenolate mofetil therapeutic drug monitoring in *de novo* kidney transplant recipients

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Introduction: MMF is currently administered according to a fixed dosing regimen but accumulating data suggests that therapeutic drug monitoring (TDM) of its active metabolite mycophenolic acid (MPA) may optimize its efficacy and tolerance. The aim of this randomized trial conducted in 11 French transplantation centres was to investigate the effectiveness of MMF monitoring based on patients' global exposure to MPA.

Methods: A total of 137 kidney transplant recipients were included. They received a classical immunosuppressive regimen combining basiliximab, CsA, MMF (2 g/day until day 7) and steroids. After D7, the fixed dose group (FD, 67 patients) carried on receiving 2 g/day MMF while the concentration controlled group (CC, 70 patients) received a MMF dose adjusted to the area under the concentration curve ($AUC_{0-12\,h}$) of MPA with a target of 40 h.mg/L. Plasma MPA was determined using HPLC-UV in all centers and MPA $AUC_{0\text{-}12\,h}$ was calculated using a Bayesian estimator and a 3-point limited sampling strategy on days 7 and 14, and months 1, 3, 6 and 12 in both groups (results not reported to the physicians in the FD group).

Results: A total of 130 patients (65 in each goup) could be evaluated. The mean $AUC_{0\text{-}12\,h}$ was significantly higher in the CC group on day 14, M1 and M3 (34 vs. 27, 45 vs. 34 and 45 vs. 37 h.mg/L; $P < 0.01$) due to an increased daily dose of MMF to reach the predefined target. At M6, MPA AUCs and MMF daily dose were similar in both groups. There were significantly less treatment failures (combination of death, graft loss, acute rejection episodes, MMF discontinuation) in the CC group ($n = 19$ vs. 31, $P = 0.03$), mainly due to less clinical acute rejection episodes ($n = 8$ vs. 20, $P = 0.01$). Patient and graft survival, as well as the incidence of adverse events were identical in the two groups. Interestingly, biopsy proven acute rejections were also significantly less in the CC group ($n = 5$ vs. 16, $P = 0.02$) and Cox regression modelling confirmed that only MMF monitoring was a significant factor linked with freedom from rejection.

Conclusion: MMF TDM using Bayesian estimation is feasible, effective and safe in *de novo* renal transplant patients. It leads to an increased MMF dose up to M6, significantly less rejection episodes and no significant increase in the incidence of infections, GI or hosphorgical side effects.

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Population design evaluation and optimisation for multiple responses models: application to the pharmacokinetics of AZT and AZT-TP

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Introduction: Population pharmacokinetic (PK) and pharmacodynamic (PD) modelling is today an essential tool for drug development and for a better use of drugs. Multiple responses models are increasingly used in those population analyses. It allows to jointly describe observations coming from different types of measurements such as, the simultaneous modelling of both PK and PD data or the PK data of a parent drug and its metabolite. These analyses rely on nonlinear mixed effects models. In this context, tools for design optimisation and evaluation become necessary. Indeed, the precision of parameter estimates depends on the choice of the design to collect the data. The objective of this study is to propose an approach to evaluate and optimise population designs in the context of multiple responses models based on the Fisher information matrix. We then illustrate this approach for design evaluation and optimisation of prospective studies including the joint population PK modelling of azidothymidine (AZT) and of its active metabolite AZT-TP.

Methods: We first extend the expression of the population Fisher matrix for multiple responses models. We implement this expression in an extension of PFIM, a R function for population designs evaluation and optimisation. We evaluate by simulation the relevance of the predicted standard errors (SE) computed by PFIM by comparison to the empirical ones obtained by estimation with NONMEM (FO and FOCE method). To do that, we simulate 1000 data sets of 100 subjects using a PKPD model example. We also compute bias and RMSE for both methods. Using data from the clinical trial COPHAR2–ANRS 111, we perform the first population PK joint modelling of AZT/AZT-TP with NONMEM. We then use the extension of PFIM to optimise several designs.

Results: The predicted SE computed by PFIM are very similar to the empirical ones obtained with the FOCE method. By contrast, the empirical SE for the FO method are bigger than the SE predicted by PFIM and those obtained with FOCE method. Moreover, we show large bias of estimation using the FO method especially for the C50 parameters which is the link between PK and PD. An optimal design for the joint population analysis of AZT and AZT-TP is determined with only, for each subject, three measurements of AZT concentrations and two measurements of AZT-TP concentrations. This design allows precise parameter estimates with the same number of subjects as the empirical design of four measurements for both AZT and AZT-TP, but with less samples in each patient.

Conclusion: We extend PFIM to population designs optimisation and evaluation for multiple responses models. We show the relevance of this tool and we illustrate its usefulness in the context of AZT/AZT-TP PK modelling.

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Lipid-lowering drug exposure in patients developing a chronic muscle disease. A 2-year retrospective study

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Introduction: Some case reports suggest that lipid-lowering drugs, especially statins, could induce or favour the clinical expression of chronic muscle diseases. However no pharmaco-epidemiological study has assessed this question. The present study was performed to describe the frequency and the characteristics of the exposure to statins and fibrates before the occurrence of a chronic muscle disease after the age of 50 years.

Methods: This was a retrospective study of chronic primary muscle disease cases diagnosed in Toulouse University Hospital between January 2003 and December 2004. Patients were identified through the databases of the Pathology Laboratory and of the hospital medical discharge (PMSI) for hospitalisations corresponding to a list of 15 specific ICD-10th diagnostic codes. We then selected the patients fulfilling the following inclusion criteria: 1) age above 50 years at the onset of the symptoms 2) presence of muscle symptoms (cramps, fatigability or loss of strength, muscle pain, atrophy or hypertrophy) 3) at least two criteria among: elevated creatine hosphor-kinase (CPK); a myogenic electromyogram; muscle biopsy abnormalities diagnostic for or evocating a muscle disease; muscle degeneration proved by CT-scan or MRI 4) a duration of the disease longer than 1 year at its diagnosis or the introduction of a specific drug for inflammatory, metabolic or degenerative muscle disease 5) primitive muscle disease. We excluded patients with vasculitis, polymyalgia rheumatica, lupus, sicca syndrome, sarcoidosis, amyloidosis, infectious or toxic myopathy, thyroid disease-related myopathy. Exposure to statins was determined by the analysis of the medical charts and by calling the patient's family doctor. We then compared the exposure to statins and fibrates of the patients living in our region with that of controls selected through the Midi-Pyrénées Health Insurance System. For each patient, five controls were matched on gender, birth year and location. Comparison of percentages (Chi-square test) and odds-ratio for the risk to develop chronic muscle disease were calculated using the EPI-INFO software.

Results: Thirty women and 15 men fulfilled the criteria of the study. The mean age was 65.8 (SD: 9.4) years. Twenty-six patients suffered from dermatomyositis or polymyositis, 15 from genetic myopathy, 4 others from an unclassified disease. Nineteen patients (42%) were exposed to statin and two (4.4%) to fibrate at the onset of their muscle symptoms. The median duration of the prescription was 31.8 months (range: 2–72). Concerned drugs were pravastatine (48%), atorvastatine (21%) simvastatine (21%), fluvastatine (5%) and cerivastatine (5%). Fifteen out of 37 patients (40.5%) living in Midi-Pyrénées were exposed to statins, and one (2.7%) to fibrates. The prevalence of exposure to statins, but not to fibrates, was higher in patients than in the control population (23.8% exposed to statins, $P < 0.05$; 7% exposed to fibrate, $P > 0.30$). The estimated odds ratio of exposure to statins in patients developing chronic muscle diseases was 2.39 [IC95%: 1.16–4.94].

Conclusion: Patients developing chronic muscle diseases after the age of 50 identified in this study present a high level of statins' exposure, suggesting an increased risk of chronic muscle diseases. Further studies are needed to confirm this hypothesis.

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Is it a link between nonsteroidal antiinflammatory drugs and severity of bacterial infections: a case control study?

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Introduction: Life-threatening infections have been described following NSAIDs use and an association has been shown between NSAIDs exposure during varicella and necrotizing fasciitis. We aimed to investigate whether NSAIDs use during evolving bacterial community-acquired infection in adults might lead to severe sepsis or septic shock.

Methods: Prospective case-control study multicenter in eight French intensive care units (ICU). Cases were all patients more than 15 years old, admitted to an ICU, for a severe sepsis or septic shock due to a bacterial community-acquired infection. Controls were patients admitted to a medical unit for a non severe sepsis. Each case was matched to one control by age, site of infection and presence of diabete. The observational period was between the couple of days before the onset of infection and the beginning of severe sepsis or septic shock for cases. Using of NSAIDs including coxibs and aspirin (>350 mg) was considered if by general route, whatever duration (acute or chronic administration), and dosage. The main criterion was the proportion of patients using NSAIDs during the observational period. The study was planned as considering a 20% exposition rate among controls and an odds ratio of two.

Results: We analysed 152 pairs of cases and controls. Main sites of infection were lung (47%), urinary tract (20%) and skin and soft tissues (10.5%). The use of NSAIDs did not differ between cases and controls (27% vs. 28%) in the overall population or in the different sites of infection). There was still no difference whatever duration of exposure and if NSAIDs treatment was acute or chronic. However in cases, the median delay before effective antibiotherapy was twice more in those exposed to NSAIDs than in non-exposed cases (6 days [CI 3–7 days] and 3 days [CI 2–3 days], $P = 0.05$). It suggests that NSAIDs delay the effective antibiotic therapy; probably by masking the progression of disease due to the suppression of the inflammatory response they induced. This data is very important to consider as delay of diagnosis and effective antibiotherapy has been shown as a main risk factor for mortality.

Conclusion: Our study was the first case control study performed in adults with various bacterial infection or severe sepsis and septic shock. Our results failed to support the hypothesis that NSAIDs during bacterial community-acquired infection increase the risk of severe sepsis or septic shock. Nevertheless, in severe sepsis or septic shock NSAIDs delay the effective antibiotherapy suggesting they mask the symptoms. Further studies are required to confirm that NSAIDs delay antibiotherapy.

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In-hospital mortality in diabetic patients with acute myocardial infarction (AMI) according to use of sulfonylureas (SU) as chronic pre-admission antidiabetic treatment

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Introduction: The cardiac effects of SU and the potential influence of B-cell selectivity in patients sustaining an AMI are not settled. To assess in-hospital mortality according to use of preadmission SU in diabetic patients admitted for AMI in a nationwide French registry.

Methods: The FAST-MI registry included consecutive patients admitted for ST-elevation (STEMI) or non-ST elevation myocardial infarction <48 hours of symptom onset, in 220 French intensive care units over 2 months from October 2005. In-hospital outcome of all 1268 known diabetic patients was assessed according to pre-admission treatment: no antidiabetics (Gr0), sulfonylureas (SU+), other medications (SU-).

Results: Groups (Gr0 vs. SU+ vs. SU-) were comparable on age (69 ± 13, 69 ± 11, 70 ± 11 years), but differed regarding history of MI (22%, 20%, 29%; $P < 0.001$), renal failure (9%, 4%, 13%; $P < 0.001$), and AMI type (STEMI: 51%, 47%, 38%; $P < 0.001$). Admission blood glucose was 11 ± 6, 12 ± 6,

11 ± 5 mmol/L, respectively. Use of primary coronary angioplasty in STEMI patients was comparable in the three groups (37%, 39%, 35%), IV thrombolysis was used in 23%, 20%, 18% ($P = 0.04$).
Mortality was 8.7% in Gr0, 4.1% in SU+, and 7.85% in SU- patients ($P < 0.025$). Among SU+, mortality was higher with glibenclamide (7.8%) than with gliclazide or glimepiride (3.0%) ($P = 0.03$).
Multiple logistic regression analysis was used to take into account potential confounders and determine independent prognostic variables. Three parameters were found to be independent mortality predictors: age (OR: 1.05/year increase; 95%CI: 1.03–1.08, $P < 0.001$), STEMI (OR: 2.10; 95%CI: 1.32–3.34, versus NSTEMI, $P < 0.001$) and preadmission treatment (Gr0: OR 1.07, 95%CI: 0.58–1.99, SU+: OR 0.51, 95%CI: 0.29–0.88, $P = 0.017$, versus SU-).
Conclusion: In-hospital outcome of diabetic patients sustaining an AMI appears better in patients on chronic SU treatment than in those receiving other medications. These data also suggest improved outcomes with newer selective SU (gliclazide, glimepiride) than with older SUs such as glibenclamide.

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Pharmacodependence and antidepressors: an epidemiological discrimination

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Introduction: Reports monitoring epidemiology or reports from experts do not mention antidepressors, apart from amphetaminergiques molecules, as being implied in addictive behaviours; yet sporadic reports notified to center for evaluation and information on pharmacodependence (CEIP) spot increases of posology for certain antidepressors. This is why we decided it was necessary to sort out overconsumptions which result from the prescribing doctor's decision to seek a better efficiency or different properties out of a use of the drug beyond approval norms, from pharmacodependences which are the result of a deliberate overconsumption on the part of the patient, and a compelling search for the drug.
This paper presents an epidemiologic comparison of overconsumption characteristics of two antidepressors, milnacipran (whose dependence potential is unknown, but for which CEIP's were reported overconsumption notifications), and tianeptine (whose abuse and dependence potentials are fully documented). It shows that it is possible to determine a specific trace for a drug which has an abuse or misuse potential.
Methods: Pharmaco-epidemiologic study of phase IV utilisation data generated by the data base of the Caisse Régionale d'Assurance Maladie des Pays de la Loire (regional office of health insurance of Pays de la Loire). From the overconsuming patient file on one hand, and the non-overconsuming patient file on the other hand, descriptive and comparative analysis are performed. Logistic regression models were then applied allowing identification of the variables which explain overconsumption.
Results: We identified variables which can suggest, in the case of tianeptine a potential abuse or pharmacodependence, and in the case of milnacipran a seriousness of the pathology or an inefficiency of the treatment.
Logistic regression models had a discriminating capability which exceeds 90% and showed an excellent adjustment (Hosmer and Lemeshow goodness-of-fit test: $P = 0.99$ for milnacipran et $P = 0.86$ for tianeptine, a large P-value indicating a good model fit). Selected variables were different for both antidepressors and provided a good characterization of overconsumption modalities: insufficient efficiency/seriousness of the pathology in the case of milnacipran OR = 11.1 (3.3–36); abuse/misuse for tianeptine OR = 10.8 (3.9–29.6).
Conclusion: A new method of analysis makes it possible to provide an explanation of posology increases.
A new approach of abuse and dependence practices is made possible with the development of new tools, complementary to the official ones, for the evaluation and analysis of drug misuse.

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Pain in Parkinson's disease – pharmacoepidemiological research into the consumption of Analgesic drugs

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Introduction: Patients with Parkinson's disease (PD) frequently experienced painful sensations which could be related to neuropathic pain or/and musculoskeletal pain. Nevertheless, there are no epidemiological data about frequency of pain in PD. The aim of this study was to describe the frequency of pain in PD patients using analgesic prescription histories and to compare with that observed in the general population and two other samples of painful patients: diabetics (suffering from neuropathic pain) and osteoarthritis patients (suffering from musculoskeletal pain) in two regions in France [Midi-Pyrénées (MP) and Provence-Alpes-Côte d'Azur-Corse (PACA)].
Methods: PD patients were identified from health insurance system databases during the last trimester of 2004. We compared drug use of this sample during 2005 to randomly selected controlled population (containing general population, diabetic and osteoarthritic patients) matched on demographic factors. Pain was defined by the use of at least one analgesic drug and chronic pain by more than three deliveries, or more than 90 DDD of one specific analgesic drug. Analgesic drugs were opiates (N02A), other analgesics (N02B), some antiepileptics: carbamazepine, oxcarbazepine, clonazepam, acid valproic and gabapentine, two antidepressants: clomipramine and amitriptyline and non-steroidal anti-inflammatory drugs [NSAIDs] (M01A).
Results: The study included 4162 PD patients in MP and 7304 in PACA. Sex ratio H/F was 0.8 and the mean age was 77 ± 11 years. PD patients received significantly more analgesics than general population (82% vs. 77%) whatever the kind of analgesic drugs. Their global consumption of analgesic drugs was not significantly different from diabetics (82% vs. 81%). PD patients received significantly more antiepileptics and antidepressants than diabetics whereas NSAIDs were less used. Compared to osteoarthritis patients, PD patients used significantly less analgesics (82% vs. 90%) and particularly NSAIDs. Concerning chronic pain, PD patients were significantly more to receive at least three prescriptions than general population (61% vs. 53%) and to use chronically opiates, paracetamol, antiepileptics and antidepressants.
Conclusion: The prevalence of pain estimated by analgesic drug consumption was significantly more important in PD patients than in general population. The prevalence rate for pain in PD patients was similar in diabetics but less than in osteoarthritis patients. Finally, characteristics of analgesic drug use were closely related to that observed in diabetics suggesting that neuropathic pain could be predominant in PD patients.

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Adrenal insufficiencies associated with inhaled corticosteroids: an under recognised event?

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Introduction: Owing to their excellent benefit/risk ratio, inhaled corticosteroids (ICS) are widely used for the treatment of chronic asthma. Yet there exists a risk of systemic effects such as adrenal insufficiency (AI) at higher doses [above 500 µg/day in children or 1000 µg/day in adults (beclomethasone-equivalent)]. The present study aimed to give an estimated frequency and describe characteristics of AI cases in patients using ICS during the past 5 years in France.
Methods: In a retrospective cohort study, all metropolitan French pediatricians, endocrinologists, pulmonologists and intensive care physicians ($n = 11783$) were mailed questionnaires requesting information regarding cases of AI related to ICS therapy between 2000 and 2005. Patients having used systemic corticosteroids during the 3 months preceding diagnosis were excluded. Data collected were patient demographics, ICS treatment characteristics, underlying condition(s), concomitant treatment(s), results of biological investigations and AI outcome. All cases were validated by an expert committee. The French pharmacovigilance database was screened for spontaneous reports to determine the frequency of AI associated with the use of ICS, using the capture-recapture method.
Results: Forty-six cases of AI were reported. Twenty-three subjects presented with AI alone and 23 with AI and Cushing's syndrome. Biological data confirming the diagnosis were provided in 32 cases (12 children and 20 adults). ICS used were fluticasone ($n = 19$), budesonide ($n = 10$) and beclomethasone ($n = 3$). In 80% of the cases, the ICS was used at high doses (>500 µg/day beclomethasone-equivalent in children and >1000 µg/day in adults). A potential drug interaction was found in 11 cases. Thirteen cases were found in the French pharmacovigilance database, one of which was common with the questionnaire survey. The capture-recapture method provides an estimation of 598 cases [95% CI (551–648)] of AI associated with the use of ICS for the 2000–2005 period in France.
Conclusion: Adrenal insufficiency induced by inhaled corticosteroids has an estimated frequency greater than 100 cases per year for about 10 M ICS units sold in France. Physicians should be advised that doses of 500 µg/day beclomethasone-equivalents in children and 1000 µg/day in adults should not be exceeded. When a high dose of ICS is clinically required, an adrenal risk should be considered.

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Leisure activities and psychotropic drug use in a French community-dwelling elderly population

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Introduction: The use of psychotropic drugs by older persons has been a subject of interest for several decades. The aim of this study was to analyze the relationship between social and leisure activities and the use of psychotropic drugs among elderly people living at home.
Methods: We studied 5008 community-dwelling elderly persons aged 65 years or more included in the 3C Study (France). Information was collected using a questionnaire administered to the respondents by trained psychologists during face-to-face interviews at home and a self-administered questionnaire. Baseline examination included socio-demographical characteristics, drug exposure as well as social and leisure activities (including mental activities such as cross-words, physical activities, productive activities such as gardening, recreative activities such as watching television). We classified as psychotropic drug users, subjects who reported taking at least one psychotropic drug during the month preceding the interview. The association between the use of psychotropic drugs and social/leisure activities were studied using multiple logistic regression adjusted on usual potential confounders. We calculated the odds ratios (OR) and 95% confidence interval (CI).
Results: Nearly 26% of participants used psychotropic drugs (mainly benzodiazepines, 19.87% and antidepressants 6.27%). Our analyses were adjusted on age, gender, education level, self-rated health, number of visits to the general practitioner, psychiatric symptoms and disability grade. Psychotropic drug was associated with the following activities: mental activities, none versus weekly (OR = 1.20; 95%CI 1.02–1.42), less than weekly versus weekly (1.23; 1.02–1.48); physical activities, none versus monthly (1.35; 1.16–1.56); productive activities, less than 1 hour per day versus more than 2 hours per day (1.28; 1.06–1.54), 1–2 hours per day versus more than 2 hours per day (1.26; 1.05–1.51); recreative activities, less than 2 hours per day versus more than 2 hours per day (0.84; 0.73–0.97).
Conclusion: Our results showed that psychotropic drug use in the elderly is inversely associated with active and mentally stimulating activities but associated with sedentary activities.

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Diversion of high dosage buprenorphine: impact of a prescription monitoring program and trends from 2000 to 2005

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Introduction: Despite the efficacy of the opiate maintenance program with high dosage buprenorphine implemented in France since 1995, a high level of diversion (doctor shopping, deal) of this medication has been observed since its introduction. The French Social Security System has begun a prescription to counter this problem in October 2004 to solve this problem. Records of high dosage buprenorphine deliveries are available since 2000 in the Bouches du Rhône area. We used these records to assess the evolution of HDB diversion (approached by doctor shopping indicators) from 2000 to 2005 to assess the impact of this prescription monitoring program.

Methods: We extracted all high dosage buprenorphine deliveries reimbursed by the General Health Fund during eight periods (first and second semester of year 2000, 2002, 2004 and 2005) in Bouches du Rhône area (population 1.88 million). For each of these periods, we used two indicators to evaluate the diversion of high dosage buprenorphine: doctor shopping ratio (percentage of total delivered quantity delivered obtained by doctor shopping) and doctor shopping quantities (number of DDD obtained by doctor shopping) given in thousands of DDD (kDDD). We used delivered quantity and number of patients treated regularly as indicators of access to treatment.
Results: Doctor shopping ratio increased steadily from 1st semester 2000 to 1st semester 2004 (from 14.9% to 21.7%) and then decreased (21.4% for 2nd semester 2004, 20.5% for 1st semester 2005 and 16.9% for 2nd semester 2005). Doctor shopping quantity increased steadily from 1st semester 2000 to 1st semester 2004 (from 631 kDDD to 1151 kDDD) and then decreased (1144 kDDD for 2nd semester 2004, 1092 for 1st semester 2005 and 858 for 2nd semester 2005). Number of patients treated remained stable from 1st semester 2000 to 2nd semester 2005 (2039 to 2129 patients, with a peak to 2148 in 1st semester 2002). Delivered quantity increased from 1st semester 2000 to 1st semester 2005 (from 3488 to 4347 kDDD), and decreased in 2nd semester 2005 (4034 kDDD).
Conclusion: After a four years increase of diversion for buprenorphine in Bouches du Rhône area, the beginning of the prescription monitoring program of the Social Security is concomitant with a marked decrease of doctor shopping indicators. These two events are probably linked. No diminution in access to treatment is noted during the same period.

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A case of Möbius syndrome in a newborn baby after medical elective abortion failure

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Introduction: Misoprostol is one of the two medicines used for medical elective abortion. When abortion fails, misoprostol is suspected to cause teratogenic effects, in particular the Möbius syndrome. Most reports have involved anarchic use of the molecule, especially in countries like Brazil where elective abortion is not allowed.
Methods: We report a case of a child born with a Möbius syndrome following exposure *in utero* to mifepristone and misoprostol for elective abortion.
Results: A pregnant woman 28 years of age decided to stop her pregnancy with medical procedure. At the beginning of the 7th week of pregnancy she received 600 mg of mifepristone and, 2 days later, 400 µg of misoprostol. One month later, despite important metrorrhagia, an ultrasound examination showed ongoing pregnancy. The woman gave birth to a boy, after 34 weeks of pregnancy, with a left facial palsy, with microretrognathia and axial hypotonia, related to a Möbius syndrome.
Möbius syndrome is characterized by uni- or bilateral palsy of the abducens (VIth) and facial (VIIth) cranial nerves. Involvement of other cranial nerves is common, like the hypoglosseal (XIIth). Craniofacial and orofacial anomalies, and limb malformations are often associated with this disorder. The etiology of Möbius syndrome is multifactorial and remains largely unknown. The most likely hypothesis is disruption of the developing vascular system, with transient ischemia, particularly in the vertebral arteries, and foetal hypoxia. The critical period for the Möbius syndrome appears to be between 5 and 7 weeks of gestation. A teratogenic cause of Möbius syndrome has been suggested. Mifepristone alone does not appear to be involved in this mechanism, however misoprostol is clearly responsible for both vasoconstriction and vascular disruption. It has been demonstrated that oral or vaginal misoprostol administration can lead to a significant increase in Doppler-measured uterine artery resistance. Misoprostol also induces uterine contractions which, if they occur during the critical embryonic period, may cause flexion in the area of the VIth and VIIth cranial nerves which are subjected to decreased blood flow.
Conclusion: This case study shows that ineffective use of misoprostol during the first trimester of pregnancy, especially between weeks 5 and 7, is associated with a risk of Möbius syndrome in infants. Attentive care should thus be given to pregnancies after failed abortion with misoprostol administration.

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Anti tumor necrosis factor during pregnancy

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Introduction: Antibody against human tumor necrosis factor (anti-TNF) are currently used in chronic inflammatory diseases (rheumatoid arthritis, Crohn's disease, ankylosing spondylitis…) which can involved age being pregnant women. Anti-TNF drugs exposure during pregnancy are the subject of a call to the regional centers of pharmacovigilance (CRPV), so the majority are not recommended during the pregnancy and contraception is necessary during these treatments. It appears therefore essential to record any data concerning foetal exposure to these drugs. We report a follow-up of pregnancies exposed to anti-TNF drugs having been the subject of a request to the CRPV.
Methods: We analyzed pregnancies exposed to anti-TNF drugs having motivated a call to a CRPV between 1/01/2001 and 31/03/2006 and recorded in our database. Only the pregnancies with known outcome were retained in the analysis.
Results: A total of 34 questions related to a catch of anti-TNF (23 infliximab, 11 étanercept) during the pregnancy were identified.
These questions were divided into questions carrying about the risk at a woman treated by anti-TNF and wishing a pregnancy (10), pregnancies having started after the stop of the medication (8), pregnancy during father treatment by infliximab (1) and women treated during first trimester or more (15).
Our results concern only these 15 pregnancies really exposed to anti-TNF (infliximab 12, étanercept 3). Pregnancy outcome is known eight times (infliximab 6, etanercept 2) and four women received concomitant medications including azathioprine, 6-mercaptopurine, prednisone, indomethacin. Three women treated by infliximab continued the treatment during all pregnancy and three stopped it during the 1st trimester. The two women treated by etanercept stopped the drug during the 1st trimester.
These eight pregnancies exposed to anti-TNF drugs during organogenesis period led to an elective termination of pregnancy in one case (no autopsy) and seven live births without apparent malformation or neonatal morbidity except a slight hypotrophy in one infant. A normal development at 2 months and 4 months were obtained in two of these children.
These data suggest no increased adverse outcomes following exposure to infliximab or etanercept during pregnancy. However, they are insufficient to affirm foetal harmlessness in short and long term period because of their manpower and absence of centralization of all the exposed cases.
Conclusion: These data are in agreement with the others published on the malformatif risk of the infliximab. They underline the scientific and ethical need to set up a systematic follow-up of any pregnancy exposed to new drugs as much as they should not be used during pregnancy.

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Male hypofertility and drug exposure: an analysis of observations recorded by the Pharmacovigilance Centres

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Introduction: Effects of drugs on female reproductive system are a major concern. Conversely, few data concerning adverse drug reactions (ADR) in male reproductive function are available. The aim of the study was to review adverse effects related to male infertility registered in the French Pharmacovigilance database.
Methods: This study was based on spontaneous reports of adverse drug reactions submitted to French Pharmacovigilance system. All cases of 'sperm impairment', 'oligospermia' and 'male hypofertility' registered from 1985 to March 2005 were reviewed. For each report, information about age, drug exposure and characteristics of ADR (imputability, time of onset, seriousness and outcome) were collected. Suspect drugs were listed according to the ATC classification (Anatomical Therapeutical and Chemical).
Results: A total of 66 cases of male fertility impairments were spontaneously reported from 1985 to March 2005: 42 'sperm impairment', 16 'oligospermia' and 8 'male hypofertility'. Mean age of cases was 34 ± 8 years. Time to onset was 21 ± 24 months after beginning suspect drugs. Twenty per cent of the adverse drug reactions induced incapacity or prolonged hospitalization. 4.5% of the cases had improved with sequela. The mean number of drugs used was 1.7 ± 1.1. The drugs most frequently involved in male hypofertility were neurological (mainly antiepileptic drugs) (31.6%), antineoplastic (18.4%) and gastrointestinal (mainly intestinal anti-inflammatory and antacids) (15.8%) drugs.
Conclusion: The study shows that neurological and antineoplastic drugs, known to impair male fertility, were the most frequent drugs involved in male hypofertility. The results also suggest that various drugs (not previously listed to induce each an ADR) like gastrointestinal drugs could be involved in male hypofertility.

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Neonatal outcomes after exposure to benzodiazepine in late pregnancy

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Introduction: Possible neonatal symptoms resulting from benzodiazepine exposure in late pregnancy include the floppy infant syndrome (impregnation syndrome) and withdrawal syndrome. Their frequency has not been carefully investigated after regular maternal use for psychiatric disorders.
Methods: Data on neonates exposed to maternal benzodiazepine during late pregnancy were selected from prospective requests received by Lyon pharmacovigilance centre over the last 20 years. Exclusion criteria were (1) benzodiazepine discontinuation more than one (short- or intermediate-acting drugs) or two weeks (long-acting drugs) before delivery; (2) single exposure before delivery; and (3) patients on methadone or buprenorphine maintenance treatment.
Results: Data were available on 108 neonates born to 106 women (2 twin pregnancies) of whom 67% ingested one benzodiazepine during the whole pregnancy, and 18% were exposed to more than one benzodiazepine. Twenty five neonates were premature with 9 small for gestational age. Twenty one neonates (19.4%, 95% CI: 12.5–28.2) experienced neonatal symptoms compatible with the responsibility of maternal drug exposure. Two groups of patients were identified according to drug exposure in late pregnancy: exposure to benzodiazepines only (group I, 33 patients) and exposure to benzodiazepines and concomitant psychotropic drugs, mostly antidepressants or neuroleptics (group II, 73 patients). The incidence of neonatal symptoms in group I (34 neonates) was 5.9% (95% CI: 0.7–19.7) and included one severe withdrawal syndrome in a premature female exposed to bromazepam and alprazolam, and one benign impregnation syndrome in a full-term infant exposed to oxazepam. In group II (74 neonates), neonatal symptoms were identified in 19 neonates, but were more probably related to the benzodiazepine than any of the other drugs in 12 cases, resulting in a calculated incidence of 17.9% (95% CI: 9.6–29.2). Overall, 14 of 21 cases with neonatal symptoms were likely to be attributable to benzodiazepine exposure (13.9%, 95% CI: 7.8–22.2). Of these, four consisted of the floppy infant syndrome, three of withdrawal symptoms and seven of both types of symptoms. Seriousness was graded as severe in seven cases, moderate in three and benign in four. According to the half-life of benzodiazepine, the incidence of neonatal symptoms likely to be attributable to benzodiazepines was twice more frequent with long-acting derivatives (7/36) compared to short- or intermediate-acting derivatives (7/72).
Conclusion: Neonatal symptoms attributable to maternal benzodiazepine treatment in late pregnancy are frequent and potentially severe. Careful surveillance of the neonates is required, particularly in those exposed to multiple psychotropic drugs or long-acting benzodiazepines.

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Drug safety of thiazolidinediones in France

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Introduction: Thiazolidinediones represent a new class of drugs for type 2 diabetes. However, several cases of serious hepatic adverse drug reactions were reported with troglitazone, leading to its withdrawal in 1999. Concerning the 2 others drugs available (rosiglitazone and pioglitazone), despite some case reports of

hepatic adverse events, it remains unclear whether or not hepatic adverse drug reactions are a class effect or are related to specific properties of troglitazone. Thiazolidinediones could induce or worsen heart failure in patients at risk. The aim of our study was to investigate the profile of adverse drug reactions related to thiazolidinediones in the French Pharmacovigilance database and to investigate potential risk factors associated with this profile in comparison with other diabetic patients identified from the French Pharmacovigilance database.
Methods: We identified a population suffering from type 2 diabetes among all the records of adverse drug reactions occurred in patients exposed to at least one drug acting on glucose metabolism. We focused our attention on 3 main adverse drug reactions: heart failure, oedema and hepatitis corresponding to specific WHO-ART terms. We analysed characteristics of patients with type 2 diabetes and compared them according their exposure or not to thiazolidinediones. We performed a multivariate analysis using a backward logistic regression model.
Results: Among a total of 81 686 reports concerning patients over 18 years and recorded in the database between 01/2002 and 12/2005, we reviewed 1821 reports corresponding to patients with type 2 diabetes (2.7% of the whole database). The proportion of patients exposed to thiazolidinediones was 5.6% (102 patients), a proportion very similar to results obtained from other sources. Patients exposed to thiazolidinediones were less frequently exposed to cardiovascular drugs (suggesting that guidelines about the non use of these drugs in patients with cardiovascular disease are followed by physicians) and to sulfonylurea than other diabetic patients. The frequency of oedema and cardiac failure was significantly higher with thiazolidinediones than in other patients (6.9 and 6% versus 0.3 and 0.2% respectively, $P < 0.001$) whereas the frequency of hepatitis was similar (5.9 versus 4%, non significative). Moreover, the results of the multiple logistic regression models taking into account potential confounding factors showed that thiazolidinediones exposure remained associated with heart failure and oedema, but not with hepatitis.
Conclusion: In conclusion, the present study clearly shows that thiazolidinediones do induce oedema and heart failure in patients with type 2 diabetes despite respect of recommendations of use. By contrast, the risk of hepatic reactions with this class of drugs is similar than that of other hypoglycemic agents.

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Reported incidence and severity of suspected abacavir hypersensitivity reactions (HSR) through at least 6 weeks in a large, controlled clinical trial using a once-daily abacavir 600 mg/lamivudine 300 mg tablet (ABC/3TC FDC): The KLEAN study

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Introduction: KLEAN was designed to compare the efficacy and safety of fosamprenavir 700 mg BID + ritonavir 100 mg BID to Lopinavir/ritonavir BID both administered in combination with ABC/3TC FDC AOD. KLEAN provides the largest cohort to assess the incidence and severity of ABC HSR with ABC/3TC FDC OAD. All enrolled patients have now progressed through >6 weeks of therapy, when historically 90% of cases have appeared.
Methods: A total of 887 ART-naive, HIV+ subjects with HIV-1RNA > 1000 copies/mL and any CD4+ cell count were randomly (1 : 1) assigned to receive open-label ABC/3TC FDC plus a boosted PI for 48 weeks. Subjects developing suspected ABC HSR were allowed to substitute another NRTI and continue in the study. Descriptive results of this unplanned, interim, non-comparative analysis are reported from the GSK Safety database. Enrollment occured from 03 Jun 2004 through 07 Jan 2005. All suspected cases reported through 18 Feb 2005 are included.
Results: Cases of suspected ABC HSR were reported in 52 subjects (5.9%) completing >6 weeks of therapy. Eight (0.9%) cases were considered mild (Grade1), 24 (2.7%) were moderate (Grade2), 18 (2.0%) were severe (Grade 3 or 4) and two cases had missing severity data. Twelve cases were hospitalised; no cases were fatal; median time to onset of ABC HSR was 8 days (range: 0–35 days).
Conclusion: The incidence and onset of reported ABC HSR in the KLEAN study through a minimum of 6 weeks is consistent with previously published data on abacavir dosed once and twice daily (5.4% across 37 clinical studies; median time to onset 9 days). The severity of these cases is also within the expected range for ABC HSR in previous clinical studies (1.8–4.9% Grade 3 or 4). Any additional reports of ABC HSR will be reported with the final analyses.

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Adverse reactions to meglumine antimoniate

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Introduction: Meglumine antimoniate (MA) is the first-choice drug of treatment of leishmaniasis in Tunisia. This drug usually causes stibio-intolerance which including myalgias, arthralgias, abdominal symptoms, headache, elevation of aminotransferases and amylase, electrocardiographic changes…These events are usually seen at the first injections and aren't dose-dependant. Rarely, stibio-toxicity is observed such as renal, cardiac and hepatic failure. These events are usually seen at the end of the treatment and are dose-dependant.
The aim of our study is to analyse the type and the delay of onset of the adverse reactions associated with MA in the cases notified to the Tunisian National Centre of Pharmacovigilance.
Methods: We performed a retrospective study which concerned all suspected cases of adverse reactions due to MA, seen in the Tunisian National Centre of Pharmacovigilance between December 1990 and December 2005, and validated according to the French method of imputability of Bégaud and al.
Among 29 cases we excluded:
- One case where the data were incomplete
- Three cases where the responsibility of MA was excluded because of incompatible delay.
Results: Twenty-five cases were retained. Ten were males and fifteen were females. Their age ranged from 2 to 78 years with a median age of 34 years. All patients have received MA by parenteral way.
The type of events are: cutaneous in 14 cases, arthralgia and myalgia in three cases, cardiac failure, renal failure, haematological disorders in two cases each, seizures and anaphylactic choc in one case each. The delay between the onset of the event varied from few minutes to 3 hours in four cases and from 2 to 14 days in 12 cases. In one case the delay wasn't noted. The doses in mg/kg/day weren't available in more of the half of the cases. In patients with cardiac and renal failure, the doses varied from 900 mg/day to 3200 mg/day.
Conclusion: We observed in our study cardiac and renal failure with doses higher than the maximal daily dose recommended by the world health organisation (WHO) (maximum dose = 850 mg/day). In these cases the delay of onset of the events didn't exceed 14 days.
Analysing our data, we showed that the delay suggest a stibio-intolerance whereas, the doses and the organic failure suggest a stibio-toxicity. Separation between stibio-intolerance and stibio-toxicity seems to be not clear.

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The effect of fluconazole on cutaneous toxicity related to intraveinous sulfamethoxazole trimethoprim in HIV patients with *Pneumocystis carinii* pneumonia

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Introduction: Cutaneous toxicity is frequent in human immunodeficiency virus-infected patients treated with sulfamethoxazole trimethoprim (SMTP) for *Pneumocystis carinii* pneumonia (PCP). Risk factors of this hypersensitivity reaction are reported, including high posology, gluthation depletion, slow acetylor phenotype of N acetyl transferase 2 and reactive metabolites overproduction via cytochrome P 450 2C9 (CYP2C9) such as sulfamethoxazole hydroxylamine (SMH) and nitroso-sulfamethoxazole. In 2004, Winter et al[1] demonstrated that the production of SMH was decreased in HIV infected patients by the concomitant administration of fluconazole, inhibitor of CYP2C9. We have analyzed the effect of concomitant administration of CYP2C9 drug inhibitor on the hypersensitivity reaction occurrence in HIV patients treated with SMTP for PCP.
Methods: Fifty-nine HIV-infected patients treated between 1995 and 2005 with SMPT for PCP were included in this retrospective study. The cases were recruited in four hospitals. Characteristics of patients, data on SMPT treatment, occurrence and clinical presentation of cutaneous toxicity, and concomitant administration of CYP2C9 drug inhibitor were recorded. An exact Fisher test was performed in order to demonstrate a relationship between the occurrence of cutaneous toxicity and CYP2C9 drug inhibitor administration.
Results: Among the 29 patients who experienced a SMPT related cutaneous toxicity, five received a CYP2C9 drug inhibitor and 24 did not. The mean time to occurrence was 9.6 days. All patients improved after decreased SMPT dosage, or discontinuation. The result of the statistical analysis was in favor of our hypothesis with $P = 0.0494$.

	Cutaneous eruption	No eruption	Total
CYP2C9 inhibitor	5	12	17
Non inhibitor	24	18	42
Total	29	30	59

Conclusion: Despite the small studied population, our results suggest that fluconazole should protect HIV-infected patients from cutaneous toxicty of SMPT. This preliminary study should be completed by a further prospective and larger one about the protector effect of fluconazole in SMPT treated patients, including SMPT urinary metabolites dosage and analysis of acetylator phenotype.

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Mitoxantrone for multiple sclerosis: follow-up of 24 patients treated with very high dose

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Introduction: Multiple sclerosis (MS) is a demyelinating disease, interesting central nervous system in young people. The evolution is characterized by a relapsing remitting course, with or without neurological handicap. Mitoxantrone, first developed as an antineoplastic agent, displays activities on lymphocytes functions and central inflammatory process. So the drug was proposed in MS. The main risk is its possible delayed cardiotoxicity and leukemia. French Health authorities published warnings and decided to restrict prescriptions. Regarding these recognized risks we analyzed the outcome of high doses exposed patients in our hospital before new guidelines.
Methods: Patients with MS receiving at least one dose of mitoxantrone before new recommendations have been identified from the hospital pharmacist' prescription registry. We selected only patients who received a total dose superior to 70 mg/m^2. For each patient medical files had been screened with focus on past medical and drug history, MS diagnosis and evolution, mitoxantrone and concomitant drug treatments. Clinical, biological and imaging surveys, especially haematological and cardiological examinations have been recorded.
Results: A total of 168 patients had received mitoxantrone. 24 patients, 15 men and 9 women, (29–60 years-old) had been treated with more 70 mg/m^2. The mean follow-up was 4.3 years (from 18 months to 6 years). Past history of serious infectious disease or familial MS concerned only few patients, whereas cardiovascular risk factors were frequent with tobacco smoking (16/24), hypertension, angina pectoris and arteritis (1/24) or severe familial cardiac events history (4/24). Most of the patients received other drugs with possible cardiovascular or haematological toxicity. On monitoring, haematological changes remained moderate and transitory. Five patients (20%) experienced deterioration of the left ventricular ejection fraction (LVEF) objectived by LVEF under 50% on gamma-cardioangiography. Four patients improved after drug discontinuation. One patient with LVEF at 47% before treatment, received 96.7 mg/m^2 mitoxantrone and interferon for one year. At control LVEF was evaluated at 13% and heart transplantation was successfully performed.
Conclusion: Our follow-up shows that using high dose of mitoxantrone in aggressive uncontrollable MS seems acceptable for cardiovascular effects and leukemia, respecting initial recommendations (LEVF > 50%) and regarding past medical and concomitant drugs history. A long term survey remains necessary for delayed haematological or unexpected risks.

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Acute overdosage with milnacipran

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Introduction: Milnacipran is a dual-action antidepressant the activity of which is characterised by specific and equally potent inhibition of nor-epinephrine and serotonin reuptake at the presynaptic site.

Methods: We analysed 105 case reports of deliberate acute overdose of milnacipran from three different sources, namely cases described in the literature, cases reported to the poisons center of the Bordeaux University Hospital and cases reported to the drug safety department of the manufacturer Pierre Fabre Medicament.

Results: In 86% of the cases milnacipran was ingested with other agents, in particular with benzodiazepines and alcohol. The adverse effects of acute overdose of milnacipran alone were not severe. Up to a dose of 1000 mg the symptoms observed consisted mainly of nausea and vomiting. Also at higher doses – the highest reported acute overdose of milnacipran only was 2800 mg – the symptoms observed were benign and included mainly drowsiness, tachycardia, slight increase of blood pressure, slight depression of respiration and sweating. Cardiotoxicity such as conduction abnormalities was not an issue.

Conclusion: Severe adverse effects were only observed in patients taking an acute overdose of milnacipran in combination with other drugs. The manifestations in this setting were dependent on the type and the doses of the combined overdose and involved primarily the central nervous and the cardiovascular system. The nine cases of fatal outcome were obviously caused by the concomitantly ingested agents. No indication of an adverse interaction of acute milnacipran overdose with other drugs could be identified. On the contrary, the pharmacodynamic and pharmacokinetic properties of milnacipran speak against such a risk.

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Serotonin-induced activation of TRPV-like current in rat intrapulmonary arteries: role in cell proliferation

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Introduction: 5-Hydroxytryptamine (5-HT) is involved in numerous biological processes in vascular tissue. In pulmonary arteries, 5-HT exerts vasoconstrictor and mitogenic effects, and its implication in the pathogenesis of pulmonary arterial hypertension (PAH) is clearly established. Since the signal transduction pathways associated with 5-HT receptors remain unknown in pulmonary arteries, we investigated the effects of 5-HT on Ca^{2+} signal, membrane conductances and proliferation in rat intrapulmonary arterial smooth muscle cells (PASMC).

Methods: Microspectrofluorimetry (indo-1 as Ca^{2+} fluorescent probe) and the patch-clamp technique (in whole-cell configuration) were used to examine intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) and membrane conductance, respectively. Cell proliferation was assessed by quantitative determination of DNA synthesis using the Cell Proliferation ELISA, BrdU colorimetric method.

Results: Application of 5-HT (10 μM) leads to a biphasic $[Ca^{2+}]_i$ response, consisting in a transient phase due to mobilization of Ca^{2+} stored in intracellular compartments, followed by a sustained phase due to Ca^{2+} entry from the extracellular medium. This latter was unaffected by indomethacin (a cyclooxygenase inhibitor) and CDC (a lipoxygenase inhibitor), whereas it was inhibited by ETYA (a non-specific inhibitor of all arachidonic acid-metabolizing enzymes) and 17-ODYA (a cytochrome P450 epoxygenase inhibitor). So this 5-HT-induced sustained Ca^{2+} influx is linked to the activation of a voltage-independent noncapacitative Ca^{2+} permeable channel whose activation requires the arachidonic acid metabolization by cytochrome P450 epoxygenase. This Ca^{2+} influx was also sensitive to Ni^{2+} and ruthenium red (RR), a TRPV channel blocker, and mimicked by 4α-phorbol-12,13-didecanoate (4α-PDD), a TRPV4 channel agonist. Whole-cell recordings showed that 5-HT activated a TRPV-like current with typical characteristics: a moderately outwardly rectifying current with a reversal potential of −18 mV. Such current was sensitive to RR and mimicked by 4α-PDD. Finally, 5-HT-induced PASMC proliferation was inhibited by RR suggesting the implication of TRPV-like current in this phenomenon.

Conclusion: In conclusion, these data point out, for the first time, the activation of a TRPV-like current by 5-HT in PASMC and its potential involvement in cell proliferation. Establishment of the link between proliferation and ion channel activation may represent a molecular target for PAH treatments.

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Erythropoietin protects against acute chemotherapy toxicity in isolated rat hearts

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Introduction: The use of chemotherapeutic agents such as anthracycline or trastuzumab in oncology is limited by their cardiac toxicity. Several recent experimental studies suggest that recombinant human erythropoietin (rhEPO) can be considered as a pharmacological preconditioning agent since administration of rhEPO is known to protect against cardiac ischemic injury improving functional recovery and reducing apoptosis and necrosis. The aim of this study was thus to investigate whether preconditioning by rhEPO could protect against acute cardiotoxicity induced by doxorubicin and trastuzumab, using the isolated rat heart model.

Methods: Rats were treated with either rhEPO (5000 IU/kg, ip) or vehicle (saline). One hour later, their hearts were isolated and retrogradely perfused at constant flow. Following 20-min of stabilization, hearts were perfused during 60 min with modified-Krebs solution containing 6 mg/L doxorubicin or 10 mg/L trastuzumab. Control hearts were perfused under identical conditions but without chemotherapeutic agent. Different hemodynamic and electrophysiological parameters were assessed in hearts from the height experimental groups.

Results: Doxorubicin exposure decreased left ventricular developed pressure (LVDP approximately -40% of baseline) and increased end diastolic pressure (EDP approximately +390% of baseline) and coronary perfusion pressure (CPP approximately +70% of baseline). Incidence of ventricular tachycardia and/or fibrillation (VT-VF) was also significantly enhanced (86% vs. 0% in control group). Trastuzumab exposure increased CPP and EDP (approximately +70% of baseline for the both) without affecting LVDP. Prior rhEPO treatment significantly prevented doxorubicin-induced deleterious effects on LVDP, EDP and VT-VF incidence. RhEPO administration also prevented trastuzumab-induced deleterious effects on CPP and EDP.

Conclusion: This study shows that preconditioning by rhEPO protects myocardium against hemodynamic damage and electrophysiological injury induced by acute doxorubicin or trastuzumab exposure. Further investigations are required to elucidate the precise mechanisms involved in this effect.

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Did ACE inhibition protect skeletal muscle metabolism and exercise capacities in chronic streptozotocin-induced diabetic rats?

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Introduction: Although angiotensin converting enzyme inhibition (ACEi) is largely used to treat cardiovascular diseases, little is known concerning its effects on skeletal muscle energetic and its repercussion on exercise capacity in type 1 diabetes.

Methods: We examined the effects of two ($n = 8$) and four ($n = 8$) months treatment with ACEi (perindopril) on skeletal muscle mitochondrial function and exercise capacity in streptozotocin-induced diabetic rats (DIA), compared with untreated DIA and normal rats at two (DIA, $n = 6$ and CONT, $n = 12$) and four (DIA, $n = 8$ and CONT, $n = 8$) months. ACEi Treatment begun 3 weeks after streptozotocin injection. Rats performed endurance habituation at a speed of 10 m/min, 0% grade, 5 minutes for four successive days and then run until exhaustion. Gastrocnemius (GAS) muscles were excised and muscle fibres were permeabilized. Maximal oxidative capacities (V_{max}) and complexes I, II and IV of the mitochondrial respiratory chain were determined using glutamate-malate, succinate and TMPD-ascorbate as substrates.

Results: Exercise capacity was reduced in short- and long-term non-treated DIA animals (−67% and -64% respectively, $P < 0.001$). Oxidative capacities of gastrocnemius muscle measured with glutamate-malate (complex I) were significantly reduced in short- and long-term DIA rats compared to CONT (−33% and −34% respectively, $P < 0.05$), but did not affect mitochondrial complexes II and IV. ACEi treatment did not protect the mitochondrial function after 2- and 4-month (-40% and -45% compared to control rats, $P < 0.05$). Exercise capacity of diabetic rats was improved after 4-month treatment (+76% compared with non-treated DIA, $P < 0.05$) but was not protected after 2-month treatment (-75% compared to control, $P < 0.01$).

Conclusion: Skeletal muscle oxidative capacities were decreased in streptozotocin-induced type I diabetic rats. Interestingly, these impairments could be explained by specific complexe I dysfunctions. Even if ACEi was initiated at the beginning of the time-course of the disease, it failed to restore the muscular function but improved exercise capacity after prolonged treatment with an ACEi.

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Blood pressure is increased by conditional mineralocorticoid receptor overexpression in the endothelium

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Introduction: The mineralocorticoid hormone aldosterone (aldo) participates to the regulation of sodium reabsorption in the kidney, thus playing a key role in the control of volemia and blood pressure. However, it has been demonstrated that the mineralocorticoid receptor (MR) is also expressed in new target organs of the aldo, such as the heart and the vessel, where the role of the hormone remains unclear. Clinical (RALES and EPHESUS) and experimental studies (animal models) demonstrate clearly the importance of the aldosterone and its receptor, which would act by vascular effects in the cardiovascular diseases.

Methods: To address the specific role of the aldosterone (aldo)/glucocorticoid (gluco) or of the mineralocorticoid and glucocorticoid receptors (MR and GR, respectively) in cardiovascular pathophysiology, we generated several conditional transgenic mouse models that allow *in vivo* spatio-temporal control of MR or GR expression.

Results: *In vivo*, MR over-expression has been achieved in endothelial cells using the tetracyclin conditional system to specifically assess its role in the vessels. Myogenic response analyzed in mesenteric arteries showed an increased sensitivity to vasoconstrictors (phenylephrine, endothelin1, thromboxaneA2). *In vivo*, blood pressure in response to Angiotensin II was increased as well as awake blood pressure (tail cuff method) (mmHg: 124 ± 2 vs. 140 ± 5, controls vs. transgenic mice (DT), $n = 10$, $P < 0.01$). Echo-Doppler analysis of arterial blood flow indicated that mean velocity of the right renal artery was increased, suggesting a decrease in renal vascular resistances ($cm.s^{-1}$: 9.7 ± 0.9 vs. 14.2 ± 1.0, controls vs. DT, $n = 7$, $P < 0,004$). This was reversed by pharmacological antagonism using canrenoate ($cm.s^{-1}$: 8.9 ± 1.6 vs. 10.3 ± 1.6, controls vs. DT). ENOS mRNA expression was decreased in the kidney while those of markers of inflammation or matrix remodelling was increased (relative ratio controls vs. DT: eNOS, 0.91 ± 0.09 vs. 0.44 ± 0.04, $P < 0.02$, PAI-1,1.26 ± 0.13 vs. 2.27 ± 0.26, $P < 0.008$, osteopontin, 1.34 ± 0.22 vs. 2.33 ± 0.27, $P < 0.006$). Moreover, in the aorta, mRNA expression of several genes involved in the renin-angiotensin system, in the endothelin sytem or in the NO imbalance are significantly modulated. Renal and vascular structures were not altered in 4 months old animals as well as Na/K balance (as estimated in metabolic cages).

Conclusion: MR activation in endothelial cells only is therefore associated with increased blood pressure and altered vascular reactivity, in absence of renal collecting duct mediated MR effects.

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TrkA internalisation in human airway smooth muscle cells and over-expression of functional receptors

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Introduction: After stimulation by NGF, its TrkA receptor is internalised in neuronal cells, but this has not been evaluated in structural cells. We have here studied the 'devenir' of the TrkA receptor after stimulation by NGF (3pM) in human airway smooth muscle in culture (HASMC), and the trafficking mechanisms involved.

Methods: After cell surface biotinylation, Western blotting was performed either on biotinylated or total proteins, leading to detection of surface or total TrkA protein, respectively. The mechanism of TrkA internalisation was studied in HASMC transfected with wild–type (WT) or mutant (MUT) pincher plasmids (2 μg each), or transfected with control or clathrin siRNA (100 nM each). TrkA internalisation was also studied in presence of the clathrin inhibitors monodansylcadaverine (MDC 50 μM) or chlorpromazine (CPZ 100 μM), or of the caveolae inhibitors nystatin (30 μg/mL) or filipin (5 μg/ml). TrkA degradation was studied in presence of the lysosomes inhibitors NH_4Cl (10 mM) or chloroquine (20 μM), or in the presence of the proteasome inhibitors MG132 (10 μM) or lactacystin (20 μM). TrkA mRNA was quantified by qPCR (Light-cycler®). HASMC proliferation was studied by the XTT technique over 4 days of NGF treatment.
Results: NGF induced TrkA internalisation in HASMC (−89 ± 2% in surface TrkA at 15 min, $P < 0.001$). This internalisation increased in cells transfected with WT pincher (+40 ± 3%, $P < 0.001$), whereas it decreased in MUT pincher transfected cells (−41 ± 4%, $P < 0.001$). TrkA internalisation was partially blocked in clathrin siRNA transfected HASMC (47 ± 7% inhibition, $P < 0.001$), control siRNA having no effect, and was also partially inhibited by MDC and CPZ (58 ± 1 and 51 ± 3% inhibition respectively, $P < 0.001$). Immunofluorescence revealed clathrin recruitment to the cell membrane and co-immunoprecipitation showed TrkA/clathrin interactions. Caveolae inhibitors had no effect. NGF also induced TrkA degradation, starting at 1 h of stimulation, and being total at 5 h. Degradation was abolished by pre-incubation with lysosome inhibitors, the proteasome inhibitors remaining without effect. Functional TrkA receptors were re-synthesised, and re-expressed at the cell membrane at 10 h of NGF treatment. During a 4-days NGF treatment, TrkA protein re-expression progressively increased, reaching a 2.1-fold increase at D4 ($P < 0.01$), in parallel with a progressive increase in NGF-induced HASMC proliferation (+13 ± 2% at D4; $P < 0.001$).
Conclusion: NGF-induced activation of the TrkA receptor in HASMC induces its internalisation, through mechanisms involving clathrin and the pincher protein. This leads to TrkA lysosomal degradation followed by overexpression of functional TrkA receptors at the cell membrane. This may have physiological and pathophysiological consequences in the airways.

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Pharmacological modulation of PKR and mTOR/p70S6 K signaling pathways in a way of neuroprotection from Aβ neurotoxicity
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Introduction: Alzheimer's disease is a neurodegenerative disorder of the central nervous system characterized by two major lesions: senile plaques composed of accumulated β-amyloid (Aβ) peptide and intraneuronal neurofibrillary tangles, associated to neuronal death. Protein synthesis is modulated by different factors including the PKR (double-stranded RNA-activated Protein Kinase) and the mTOR (mammalian Target Of Rapamycin) /p70S6 K signaling pathways. mRNA translation is altered in the brain of Alzheimer's Disease patients. Moreover, our previous studies demonstrated that the initiation of translation is downregulated in different cellular and animal models and in human lymphocytes of Alzheimer's Disease patients with an activation of PKR and an inhibition of mTOR/p70S6 K pathways. These alterations of translation initiation could represent possible targets for therapeutic strategies in Alzheimer's Disease. The goal of the present study was to examine whether pharmacological decrease of the enzymatic activity of PKR or increase of the enzymatic activity of mTOR/p70S6 K can afford neuroprotection in a cellular model of extracellular Aβ neurotoxicity.
Methods: Human (SH-SY5Y) neuroblastoma cells differentiated into mature neural cells were treated first with different concentrations of PKR inhibitors or mTOR/p70S6 K activators during various times and secondly exposed to 20 μM aggregated Ab1-42 peptide. The total and phosphorylated forms of PKR (Thr446/Thr451), mTOR (Ser2448) and p70S6 K (Thr389) were analyzed by western blottings. Apoptosis was assessed by the dosage of activated caspase-3.
Results: Inhibition of PKR phosphorylation with two PKR inhibitors: a chemical compound C16 or a peptide, PRI, is able to completely reverse cellular apoptosis induced by Aβ peptide for the highest concentrations of molecules in SH-SY5Y. In the same time, the C16 compound markedly reduces the level of phosphorylated PKR in the cells.
Concerning the mTOR/p70S6 K pathway, the treatment with three potent activators: a μ-opioid receptor agonist, DAMGO, a branched-chain amino acid, L-leucine or a selective agonist of group I metabotropic glutamate receptor, DHPG causes an increased phosphorylation of mTOR and/or p70S6 K kinases in the same cellular model but this activation did not prevent the induced Aβ-cell death.
Conclusion: These results suggest that PKR plays an important role as apoptotic pathway and mTOR as cellular survival pathway in Aβ toxicity and compounds able to inhibit PKR activation could represent a possible way to protect cells from Ab toxicity.

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Substrate oxidation during exercise and muscle oxidative capacities in type 2 diabetes: effects of exercise training
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Introduction: It is now well admitted that abnormalities in skeletal muscle lipid metabolism play a key role in the pathophysiology of insulin resistance and the development of type 2 diabetes. It has also been reported in this pathology that an impaired functional capacity of mitochondria is certainly involved in perturbations of both glucose and fatty acid metabolism and that a reduction in the activity of marker enzymes of oxidative pathway correlates with the severity of insulin resistance. The aim of this study was first to compare whole body substrate oxidation and muscle oxidative capacities between type 2 diabetic patients (D) and healthy overweight subjects (C) matched for age, sex and physical activity, and then to investigate in D the effects of an individualized training program.
Methods: Eleven type 2 diabetic patients (D) and ten control subjects (C), all in overweight, participated to the study. An Intravenous glucose tolerance test to determine insulin sensitivity (SI), a submaximal exercise test for determination of fat and carbohydrate oxidation and a muscle biopsy for investigation of muscle oxidative capacities (mitochondrial respiration and enzyme activities) were performed in both groups and then repeated in D after 10 weeks of an individualized training program performed at an intensity corresponding to the maximal rate of fat oxidation (Fat_{max} point).
Results: Before training, SI and the oxygen consumption (VO2) peak were significantly higher in C than in D ($P < 0.01$) whereas fat and carbohydrate oxidation, mitochondrial respiration and enzyme activities (citrate synthase: CS and hydroy-acyl dehydrogenase: HADH) were not significantly different between D and C. Training induces in D a significant increase of Fat_{max} point exprimed in Watts (W) ($P < 0.05$) and the rate of fat oxidation at Fat_{max} point (fat_{ox}) exprimed in $mg.min^{-1}$ ($P < 0.05$) which provides evidence of a preferential use of fat during exercise. CS activity and mitochondrial capacity to oxidize palmitoyl-coA + carnitine/malate and pyruvate + malate were also significantly increased after training in D ($P < 0.05$). SI, VO_2 peak and HADH activity did not change. A strong relationship was found between CS activity and Fat_{max} point ($r = 0.58$, $P < 0.01$).
Conclusion: We conclude that whole body substrate oxidation capacities and muscle oxidative capacities did not differ between D and overweight C when matched for physical activity. Moreover, 10 weeks of an individualized exercise training targeted to an intensity corresponding to the maximal rate of fat oxidation improves whole body substrate oxidation and muscle substrate oxidation in D.

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Swimming training reduces and stabilizes unstable atherosclerotic plaques in apolipoprotein E knockout mice (apoE-/-) with renovascular hypertension
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Introduction: Exercise training is a deterrent of atherosclerotic cardiovascular disease. However, the effects of exercise on unstable atherosclerotic plaque remain poorly understood.
Methods: We used ApoE-/- mice with either stable or vulnerable plaques. Mice with vulnerable plaques were generated by increasing endogenous angiotensin (Ang) II production (two kidney-1 clip, 2 K1C, renovascular hypertension model). Norrmotensive ApoE-/- mice with normal Ang II levels and stable plaques were used as controls. Nine week old ApoE-/- mice were divided into two groups: i) the exercise group, which underwent an 11-week swimming protocol (beginning 6 weeks before surgery up to 5 weeks thereafter), and ii) the sedentary group. Quantification of atherosclerosis was determined in thoraco-abdominal aorta by Oil red staining. To assess plaque vulnerability in aortic sinus, we quantified smooth muscle cell (SMC) content in the fibrous cap of the plaque (α-SMC immunostaining) and plaque inflammation (Mac-2 immunostaining).
Results: 2K1C ApoE-/- mice developed significant hypertension compared to sham operated mice. Swimming did not reduce blood pressure. Swimming exercise strongly reduced lesion extension in aortas of 2K1C and sham ApoE-/- mice as compared with sedentary mice. SMC content was significantly reduced in fibrous cap of sedentary 2K1C ApoE-/- mice in comparison to sham animals confirming the unstable phenotype of plaques in 2K1C ApoE-/- mice. Swimming training increases SMC content in 2K1C ApoE-/- as compared with the sedentary 2K1C ApoE-/- mice suggesting a better stability of these plaque. Swimming training appeared to decrease macrophage accumulation in plaque in 2K1C ApoE-/- mice compared to sedentary 2K1C ApoE-/- animals.
Conclusion: We showed for the first time that swimming training stabilizes unstable plaque in hypertensive ApoE-/- mice. This finding suggest a new benefit of exercise training in the prevention of atherosclerosis.

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Musclin gene expression is strongly related to fast-glycolytic phenotype
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Introduction: Musclin has recently been described as a muscle-derived secreted peptide, responsive to both food deprivation (drastically decreasing mRNA levels) and insulin (increasing mRNA levels) *in vivo*. *In vitro*, musclin induced insulin resistance in C2C12 cells. Because muscle fibers display very different metabolic properties and insulin sensitivity, we tested the hypothesis that musclin expression could depend on fibre type. Moreover, whether changes in muscle phenotype induced by either muscle inactivity (i.e. unweighting) or increased muscle activity (i.e. physical training or muscle overload) affect musclin gene transcription has never been examined.
Methods: Musclin mRNA levels where measured using real-time RT-PCR and myosin heavy chain (MHC) distribution was assesd by SDS-PAGE electrophoresis. We first measured musclin transcription in soleus, plantaris and white gastrocnemius muscles of control adult rats. Then we studied musclin mRNA expression in isolated plantaris fibers previously classified according to their MHC isoform content (I, IIa, IIx or IIb). Transition of muscle phenotype was obtained by hindlimb suspension (slow-to-fast transition in soleus muscle) or overload (fast-to-slow transition in plantaris muscle), musclin transcription was measured

and compared to control animals. Finally, we studied plantaris musclin transcription in response to acute treadmill exercise or endurance training (1, 2 and 10 weeks).
Results: Musclin mRNA was detected at high levels in gastrocnemius and plantaris muscles but only as traces in soleus muscle. The single fiber analysis showed that musclin was produced by myofibers themselves, almost exclusively in type IIb fibers. Slow-to-fast transition in soleus phenotype induced by hindlimb suspension increased musclin mRNA levels. In contrast, fast-to-slow transition led by muscle overload decreased musclin mRNA levels. Finally, neither acute exercise nor exercise training modified musclin transcription in plantaris muscles.
Conclusion: Musclin transcription occurs in muscle fibers themselves and is strongly related to fast-glycolytic phenotype. This finding reveals important new information on the interaction between fiber type and musclin expression.
Major muscle phenotype transitions such as induced by unweighting or muscle overload are associated with changes in musclin expression. These results integrate musclin as a new component of muscle phenotype malleable to physiological interventions such as changes in contractile activity and/or mechanical load imposed to skeletal muscle.
Taken together with previous studies, our findings indicate that musclin could play a role in the resistance of fast-glycolytic type IIb myofibers to the insulin-stimulated glucose uptake. Further studies are warranted to determine the exact mechanisms by which musclin could contribute to insulin resistance in fast-twitch glycolytic fibers.

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Cardiovascular risk of recombinant human erythropoietin in trained rats with endothelial NO synthase inhibition
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Introduction: Chronic administration of recombinant human erythropoietin (rHuEPO) can generate serious side effects. RHuEPO, by modulating directly endothelial function, or indirectly by increasing erythrocytose, blood viscosity and shear stress on the vascular surface, can be responsible of arterial hypertension (HTA) and arterial thrombosis. The presence of nitric oxide (NO) can protect from noxious (thrombogen and hypertensor) effects of the EPO. On these bases, we studied the cardiovascular effects of a chronic administration of rHuEPO in trained rats presenting an endothelial NO-dependant dysfunction.
Methods: Rats were treated or not with rHuEPO (100 UI/kg, twice a week, subcutaneous injection) and/or an inhibitor of the eNOS (10 mg/kg/day of L-NAME) during 6 weeks. During the same period, the rats were subject to a treadmill exercise (5 days/week, 60 min/day). The blood pressure was measured at the end of every week. At the end of the protocol, an effort test was made. After sacrifice of rats, the citrate synthase activity was measured at the soleus muscle and the vasorelaxation of the aorta to acetylcholine was studied.
Results: In the group L-NAME + rHuEPO + exercise, we observed a deterioration of the exercise endurance in rats with an important mortality (50%) during the exercise or the recovery period. The enzymatic activity of citrate synthase in the exercises groups was higher than that in the sedentary groups (this activity was not modified by the rHuEPO treatment and/or L-NAME). A severe arterial high blood pressure developed in these rats (>220 mmHg) associated to a deterioration of the NO-dependent vasorelaxation (I_{max} < 60%).
Conclusion: In conclusion, the rHuEPO affects seriously the cardiovascular function in trained rats which the activity of NO synthase is blocked and potentiates the cardiovascular risk.

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Effect of physical and mental stress on HR and HRV before and after exercise training among patients with cardiac diseases
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Introduction: Modifications of autonomic nervous activation, notably during mental or physical stress, could represent a potential risk of sudden death or ventricular fibrillation in cardiac patients. Heart rate (HR) and heart rate variability (HRV) have been used to investigate these adaptations mediated by the autonomic nervous system. Effects of exercise training on HR and HRV are well known at rest, but have been less investigated during stress. This study hypothesizes that physical and mental stress and their association, could represent a potential risk in cardiac patients, by a concomitant increase of HR and sympathetic activation, and that endurance exercise training could decrease it.
Methods: A total of 24 male patients (mean age = 51.6 ± 6.5 years) suffering from coronary artery disease (n = 12) or heart failure patients (n = 12) participated to the study. They completed two experimental sessions, before and after 4-week rehabilitation program training. During each session, a Holter ECG recording was realised during a mental stress using cognitive exercises, an acute exercise at 30% of maximal power, and their association.
Results: Both stresses and their association increased HR but had no significant effect on HRV parameters. There was a potentiation of HR increase during the association of both stresses. Exercise training had no effect on HR and HRV at rest, during mental stress and during acute exercise at the same relative intensity. Considering absolute power of exercise, which is an important factor of sympathetic activation, there is an improvement of HR response to exercise after training.
Conclusion: Both stresses could represent a cardiac risk, which is well identified by HR increase than HRV in cardiac population. The association of both stresses potentiates this risk. Cardiac rehabilitation program has a beneficial effect on this potential risk during acute exercise but not during mental stress. Neural mechanisms of these adaptations should be investigated in future studies.

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Renal hyporesponsiveness to brain natriuretic peptide: both generation and renal activity of cGMP are decreased in patients with pulmonary hypertension
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Introduction: Although natriuretic peptides are known to delay the progression to overt left heart failure, their renal efficiency decreases with time, resulting in an inability to prevent progression to terminal heart failure.
Similarly, a reduced responsiveness of pulmonary hypertensive patients (PH) has been described, but the mechanisms involved remain to be determined.
Methods: Ten patients with pulmonary artery hypertension and eight matched control subjects participated in the study.
After the baseline resting period (07: 30–10: 30 h), 10 mL/kg isotonic saline solution were infused over 30 min and the subjects remained supine for the next 180 min. Blood as well as urine samples were obtained before, and at 60, 120 and 180 min after the beginning of the saline infusion.
Sodium, creatinine and cGMP were measured in urine samples. Sodium, proteins, creatinine, osmolality, BNP, cGMP, plasma renin activity (PRA) and aldosterone were determined in blood samples.
Results: PH demonstrated an impaired ability to excrete the sodium load (22 ± 6% versus 40 ± 5%, $P < 0.05$) over the 3 h follow-up period.
Plasma BNP was significantly increased in patients (64.7 ± 13.3 ng L^{-1} versus 19.0 ± 3.1 ng L^{-1}, $P < 0.01$).
NaU/BNP was lower in patients than in controls (0.0012 ± 0.0001 versus 0.060 ± 0.0020, $P < 0.05$ for NaU/BNP).
Cyclic GMP/natriuretic peptide ratio reflects the second messenger generation for a given natriuretic peptide level. Plasma cGMP/BNP difference tended to be significant ($P = 0.07$) and excreted cGMP/BNP ratio was significantly lower in patients than in controls (0.012 ± 0.005 versus 0.197 ± 0.086, $P < 0.05$).
Conclusion: In the present study, we investigated PH patients without edema and showed that they present with an impaired renal ability to excrete an acute sodium load, compared to controls. We confirmed the low natriuretic effects of BNP and found that the excreted cGMP for a given BNP level was lower in patients than in controls. In addition, the natriuresis for a given excreted cGMP level was decreased in patients compared to controls.
These results suggest that PH patients' hyporesponsiveness to BNP may be due to both decreased renal production of cGMP and reduced renal response to cGMP.

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Abstract withdrawn

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HB-EGF promotes immune glomerular injury and renal failure in crescentic rapidly progressive glomerulonephritis through activation of the epidermal growth factor receptor
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Introduction: Heparin-binding epidermal growth factor like growth factor (HB-EGF) belongs to the epidermal growth factor receptor (EGFR) ligand family. HB-EGF is a potent mitogenic factor expressed by peripheral blood mononuclear cells including T lymphocytes, macrophages, but is also expressed by various resident cells in the kidney including podocytes, endothelial cells and mesangial cells. HB-EGF has been implicated in a wide variety of pathologies, but its role in inflammatory diseases remains unknown. The aim of our study was to investigate the role of HB-EGF and EGFR in exprerimental crescentic glomerulonephritis.
Methods: Accelerated anti-glomerular basement membrane antibody-induced glomerulonephritis was performed in SV129 mice used as controle (CT), HB-EGF deficient mice (KO) and littermates treated with erlotinib or AG1478, pharmalogical inhibitors of EGFR tyrosine kinase activity (INH). Erlonitib was administered either from the very start or 4 days after the immunization.
Results: In CT, crescents formation was accompanied by marked induction of EGFR phosphorylation within glomeruli and enhanced expression of proHB-EGF mRNA in the kidney cortex (real time PCR). When compared to CT, KO and INH mice did not display high levels of EGFR phosphorylation and were partially and significantly protected against renal lesions, since they had better renal function, assessed by urea (14.3 ± 1.0 and 10.5 ± 0.3 vs. 34.1 ± 7.2 mmol/l repectively for KO, INH and CT) levels, fewer albuminuria (8.3 ± 2.2 and 8.5 ± 2.0 vs. 24.4 ± 3.1 g/mol of creatininuria), lower percentage of crescentic glomeruli (32.3 + 7.5 and 25.0 ± 6.9 vs. 62.5 ± 7.9 %) ($P < 0.05$ to 0.001 for all items). More interesting were the same experimental therapeutic actions of delayed erlotinib administration on renal damages, cell infiltrates and renal failure. At last, we observed a consistent up-regulation of HB-EGF protein expression in glomeruli from human kidneys with crescentic rapidly progressive glomerulonephritis (RPGN) compared to a low constitutive tubular expression in normal tissues (immunochemistry).
Conclusion: These data provide evidence for the concept that immune-mediated glomerular injury leads to active and sustained pathophysiological recruitment of glomerular EGFR by HB-EGF. EGFR is here demonstrated to be involved in renal inflammation, glomerular destruction and renal failure. The therapeutic potential of specific EGFR inhibitors may be envisioned in RPGN and other inflammatory glomerulonephritis.

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Impact of ABCC2 polymorphisms on methotrexate pharmacokinetics in patients with lymphoid malignancy

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Introduction: Human multidrug resistance protein 2 (MRP2, encoded by the ABCC2 gene) is involved in the active efflux of anionic drugs such as methotrexate (MTX). A rare mutation in ABCC2 gene resulting in a non-functional MRP2 protein has been described in a patient with severe MTX elimination and subsequent toxicity. This study was conducted to assess whether more common ABCC2 genetic variants may contribute to the variability of high-dose MTX pharmacokinetics (PK) and the onset of MTX adverse events.

Methods: A prospective pharmacogenetics study was conducted in 50 adults patients (27 males; mean age: 53 ± 17 years) receiving high-dose MTX (5.13 ± 1.88 g/m^2 in a 4 hours perfusion) for the treatment of a lymphoid malignancy. MTX concentrations were measured at 24 hours (H24) and 48 hours (H48) from the beginning of MTX administration according to the usual care of patients. Two additional samples were collected for the study in the first 24 hours following MTX administration. A population PK analysis was performed using data from the first MTX course (NONMEM software). Hematologic, renal and hepatic toxicity was monitored in each patient in the weeks following MTX administration. Patients were genotyped for one promoter and four non-synonymous ABCC2 polymorphisms: C-24T, G1249A, T3563A, C3972T and G4544A.

Results: The mean plasma MTX concentration at H48 was significantly lower in patients carrying at least one −24T allele ($n = 16$) compared with other patients (0.13 ± 0.12 µmol/l versus 0.29 ± 0.16 µmol/l, $P < 0{,}005$ by Wilcoxon test). 11/16 (68.75%) of patients carrying the mutated −24T allele but only 9/34 (26.5%) of patients with the wild −24C allele presented with MTX concentrations below the toxic threshold (<0.1 µmol/l) at H48 ($P < 0.005$). A multivariate linear regression analysis (stepwise selection including age, gender, MTX dose, renal function, and ABCC2 polymorphisms) identifies the −24T allele as an independent predictor of MTX concentrations at H48 ($P < 0.01$). Preliminary population PK analysis confirms the significant influence of the −24T allele on MTX PK parameters. To analyse influence of the C-24T genotype on MTX adverse events, only the 33 patients who received MTX as a mono-chimiotherapy were retained. A non significant trend toward a lower incidence of hematologic, renal or hepatic disorders was observed in patients carrying the −24T allele compared to other patients (25% versus 40% respectively). None of the other studied polymorphism was associated with MTX pharmacokinetics in our study.

Conclusion: The ABCC2 C-24T polymorphism is associated with MTX pharmacokinetics variability. Patients carrying the −24T allele are more prone to reach MTX non-toxic levels 48 hours after administration. Finally, our results suggest that patients carrying the −24T allele may have a lower risk of developing MTX adverse events.

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Influence of induction genetic polymorphism of CYP1A2 on pharmacokinetic and pharmacodynamic parameters of clozapine: a pilot study

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Introduction: The atypical neuroleptic agent clozapine is widely used in the treatment of schizophrenia and other psychotic disorders. However, it is difficult to define an efficient dosage, due to large interindividual variations. This drug is mainly metabolised by CYP1A2, which activity varies in the general population because of environmental (i.e. tobacco) and genetic factors, such as the – 164 C → A polymorphism. To study the influence of CYP1A2, on pharmacokinetic and pharmacodynamic parameters, in relation with tobacco consumption, five healthy smokers homozygous – 164 A/A were included in a pilot study.

Methods: Subjects (median age: 22 years) received oral clozapine (12.5 mg) before and after smoking cessation and clozapine and its metabolite (norclozapine) plasma concentrations were measured to determine pharmacokinetic parameters. Caffeine test was used to estimate CYP1A2 activity with the paraxanthine/caffeine plasma concentrations ratio (R1). Norclozapine/clozapine plasma concentrations ratio (R2) was measured too. In parallel a visual attention test was used to estimate roughly pharmacodynamic parameters.

Results: In the absence of tobacco consumption, mean clozapine AUC_{0-25} was significantly increased (252.7 ± 11.8 vs. 397.6 ± 108.6 ng.mL^{-1}.h; $P = 0.036$). Mean clozapine Cmax was also significantly increased (48.3 ± 18.1 vs. 29.4 ± 11.8 ng.mL^{-1}; $P = 0.041$). By contrast, no significant difference on norclozapine pharmacokinetic parameters was observed after smoking cessation. The R1 ratio was significantly decreased after smoking cessation (0.91 ± 0.48 vs. 0.39 ± 0.19; $P = 0.041$). By contrast the R2 ratio was no significantly decreased after smoking cessation (0.31 ± 0.08 vs. 0.25 ± 0.04; $P = 0.345$). Mean reaction time was significantly increased after smoking cessation (303.56 vs. 393.3 ms; $P = 0.043$).

Conclusion: Despite the small number of subjects included in this pilot study, results indicate that subjects exhibiting an homozygous genotype – 164 A/A display a high CYP1A2 inducibility which could modify significantly clozapine pharmacokinetic and pharmacodynamic parameters. This polymorphism together with tobacco consumption should be now taken into account before introduction of a clozapine treatment in order to prevent an under dosage in smoking patients.

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Association of interleukin 10 genetic polymorphisms with acute graft rejection in renal transplantation

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Introduction: Different pro-inflammatory and anti-inflammatory cytokine gene polymorphisms have been implicated in renal graft outcome, but this effect is still controversial.

The primary objective was to determine the influence of interleukin 10 (*IL10*) genetic polymorphisms on the risk of acute renal graft rejection during renal transplantation.

Methods: We report the results of an ongoing pharmacogenetic study from Transgene study, a cohort of renal graft recipients from 1996 and 2006. We studied 242 renal graft recipients who were genotyped for single nucleotide polymorphisms: IL-10 (positions −1082, −819, −592). *IL10* haplotypes were constructed with PHASE program and were correlated with acute graft rejection.

Results: In our study group, 209 renal graft recipients out of 242 were of Caucasian origin. Within the Caucasian patients, the C allele of *IL10* C-592A SNP had a protective effect against acute graft rejection compared to the A allele ($P = 0.04$). Carriers of ATA *IL10* haplotype were found to be at risk of acute rejection compared to ATA non carriers ($P = 0.04$).

Conclusion: IL10 C-592A genetic polymorphism appeared in our study to play a role in acute graft rejection as well as carriers of IL10 haplotype ATA. These results need to be confirmed in larger clinical studies in the future.

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***In vitro* comparison of the influence of the cyp 3A5*3 single nucleotide polymorphism on the hepatic clearance of three immunosuppressive drugs: everolimus, sirolimus and tacrolimus**

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Introduction: The immunosuppressive drugs (IS) tacrolimus, sirolimus and everolimus are metabolised by the cytochromes P450 (CYP) 3A enzymes. CYP3A5 is polymorphically expressed in humans: its expression is almost fully abolished in homozygous carriers of the mutated *cyp3A5*3* allele. Several clinical studies have evaluated the influence of *cyp3A5*3* polymorphism on tacrolimus and sirolimus exposure or dose requirement. No data are currently available for everolimus. In this study, we compared *in vitro* the activity of CYP3A4 and 3A5 and the influence of *cyp3A5*3* on the hepatic metabolism of tacrolimus, sirolimus and everolimus.

Methods: IS (100 µg/L) were incubated with recombinant CYP3A4 and 3A5 (rCYP, 10–75 pmole/ml, $n = 2$) as well as two pools of human liver microsomes (HLM, 0.1–0.2 mg/mL, $n = 3$) from patients carrying the *cyp3A5*3/*3* ($n = 28$) or the *cyp3A5*1/*3* genotypes ($n = 5$). Incubations (600 µL) were performed at 37°C and aliquots sampled at 0, 2, 5, 10, 15, 20 and 30 minutes. IS determination was performed using turbulent-flow chromatography tandem mass spectrometry. The intrinsic clearance (Cl_{int}) of IS depletion was estimated using the *in vitro* half-life method.

Results: Large differences were found in the Cl_{int} of IS in presence of rCYP3A4 and rCYP3A5. The rCYP3A5/rCYP3A4 Cl_{int} ratio was 2.6, 0.3 and 0.6 for tacrolimus, sirolimus and everolimus, respectively. The Cl_{int} of tacrolimus was 1.5-fold higher for *cyp3A5*1/*3* microsomes than *cyp3A5*3/*3* HLM. In contrast, *cyp3A5* genotype have no influence on the Cl_{int} of everolimus and sirolimus by HLM. For both HLM and rCYP, the Cl_{int} of everolimus was dramatically lower than those of other IS.

Conclusion: This study showed that CYP3A5 do not similarly contribute to the metabolism of IS at the hepatic level. However, the intestine mucosa could greatly contribute to their overall metabolism. The study of the influence of *cyp3A5* polymorphism of the intestinal metabolism of IS is ongoing in our laboratory.

	Cl_{int} (µL/pmole CYP[a] or mg protein[b]/min)		
	Tacrolimus	Sirolimus	Everolimus
rCYP3A4[a]	3.32	2.36	0.22
rCYP3A5[a]	8.49	0.64	0.13
*cyp3A5*1/*3* HLM [b]	1143 ± 228	1277 ± 63	334 ± 47
*cyp3A5*3/*3* HLM [b]	747 ± 112	1265 ± 223	292 ± 41

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Prostanoids contribute to cutaneous active vasodilation in humans

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Introduction: The specific mechanisms by which skin blood flow increases in response to a rise in core body temperature via cutaneous active vasodilation are poorly understood. The primary purpose of this study was to determine whether the cyclooxygenase (COX) pathway contributes to active vasodilation during whole body heat stress (*protocol 1*; $n = 9$). A secondary goal was to verify that the COX pathway does not contribute to the cutaneous hyperemic response during local heating (*protocol 2*; $n = 4$).

Methods: For both protocols, four microdialysis fibers were placed in forearm skin. Sites were randomly assigned and perfused with (i) Ringer solution (control site); (ii) ketorolac (KETO), a COX-1/COX-2 pathway inhibitor; (iii) NG-nitro-L-arginine ethyl ester (L-NAME), a nitric oxide synthase inhibitor; and (iv) a combination of KETO and L-NAME. During the first protocol, active vasodilation was induced using whole body heating with water-perfused suits. The second protocol used local heaters to induce a local hyperemic response. Red blood cell flux (RBC flux) was indexed at all sites using laser-Doppler flowmetry, and cutaneous vascular conductance (CVC; RBC flux/mean arterial pressure) was normalized to maximal vasodilation at each site.

Results: During whole body heating, CVC values at sites perfused with KETO (43 ± 9% CVCmax), L-NAME (35 ± 9% CVC max), and combined KETO/L-NAME (22 ± 8% CVCmax) were significantly decreased with respect to the control site (59 ± 7% CVC max) ($P < 0.05$). Additionally, CVC at the combined KETO/LNAME site was significantly decreased compared with sites infused with KETO or L-NAME alone ($P < 0.05$). In the second protocol, the hyperemic response to local heating did not differ between the control site and KETO site or between the L-NAME and KETO/L-NAME site.

Conclusion: These data suggest that prostanoids contribute to active vasodilation, but do not play a role during local thermal hyperemia.

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Deleterious effects of betablockers on arterial stiffness and central pulse pressure in menopausal women: baseline findings from the CASHMERE trial

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Introduction: Beta-blockers (BB) may be less effective than other antihypertensive drugs for stroke prevention in patients with primary hypertension (ASCOT and LIFE

studies). Our study compares arterial stiffness and central PP between users (BB+) and non users of BB (BB-), among menopausal women with hypercholesterolemia and no history of CV disease.
Methods: We used the baseline data of 664 menopausal women, screened for the Cashmere study, a12-month double-blind randomized trial comparing the effects of atorvastatin (80 mg/day), versus placebo, ± with hormone therapy, on the progression of CCA-IMT and arterial stiffness. Aortic stiffness was measured by carotid-femoral pulse wave velocity (PWV); central PP and augmentation index (AI, wave reflection) were determined by applanation tonometry; carotid stiffness was calculated from relative stroke change in diameter (echotracking system) and carotid PP.
Results: BB were used in 104 women for treating headache, tachycardia, arrhythmia, and hypertension. 97% BB used were devoid of vasodilating properties. Age (60 ± 6 vs. 58 ± 5 yrs, $P < 0.0001$) and mean BP (MBP: 91 ± 12 vs. 88 ± 11 mmHg, $P < 0.0001$) were slightly but significantly higher in BB+ than in BB- ($n = 560$). After adjustment to age and MBP, BB+ had 10% higher central PP ($P < 0.0001$), 6% higher AI ($P < 0.001$), 4% higher PWV ($P = 0.04$), and 5% higher carotid stiffness ($P < 0.01$) than BB−. BB+ had 4% higher central SBP ($P < 0.0001$) than BB−, despite a non significantly higher brachial SBP only (1%, P = NS). To rule out an influence of hypertension on arterial parameters, we compared users of anti-hypertensive drugs ($n = 110$) to non users ($n = 554$). No significant difference was observed concerning the above parameters, excluding or not BB− users.
Conclusion: In menopausal women with hypercholesterolemia and no CV disease, the use of non-vasodilating BB was associated with higher aortic and carotid stiffness. These data are consistent with the results of the CAFÉ trial. Whether the deleterious effects of BB on large arteries increase the risk of CV events in women remains to be determined.

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Dexamethasone prevents impairment of endothelium-dependent relaxation in organ-cultured pulmonary arteries of mice
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Introduction: By using organ-culture method, it is possible to dissociate the influence of one factor, such as hypoxia, to the others on the vascular properties. However, endothelial functionality of artery may be disturbed by long-term culture and restricts the interest of this method. Glucocorticoids such as dexamethasone (DEX) are used to treat a wide variety of inflammatory diseases and may have protective effects on the vascular endothelium. The aim of the study was to determine whether DEX prevents endothelial dysfunction observed with the arterial pulmonary organ-culture method.
Methods: Left segments of mice extrapulmonary artery (PA) (Male C57BL6/J) were placed under sterile conditions into individual wells of 8-well culture plates containing culture medium (D-MEM-HEPES supplemented with 1% penicillin-streptomycin, 1% Na pyruvate, 1% non essential acids). Organ culture plates were placed in a humidified incubator at 37°C under 5% CO_2 in air. The organoid rings were maintained in culture for 7 days in the presence or absence of DEX (1 or 10 μM). Then, contractile responses to 80 mM KCl and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) were assessed using a wire myograph and compared to responses from fresh dissected PA (control). The relaxant effect of acetylcholine (ACh), ionophore A23187 and sodium nitroprusside (SNP) was studied in PA from culture and control group precontracted with 10 μM of $PGF_{2\alpha}$.
Results: Contractile response to KCl was unchanged after 7 days of culture. Addition of DEX in culture medium did not modify the contractile response of PA to KCl. No change in maximal contraction to $PGF_{2\alpha}$was observed by culture conditions despite a significant increase in sensitivity in the culture group (EC_{50}: 2.6 ± 0.7 versus 8.7 ± 1.7 μM, $P < 0.001$, in culture and control, respectively). This hypersensitivity was prevented when DEX was added in culture medium (EC_{50}: 10.0 ± 2.7 and 8.5 ± 3.2 μM, for 1 and 10 μM of DEX, respectively). Maximal relaxation to ACh was largely reduced in culture conditions compared to control (15 ± 3 versus 62 ± 3%, $P < 0.001$, respectively). A similar alteration of the relaxation was observed with the ionophore A23187 in PA from culture conditions whereas the relaxation to SNP was unchanged. Addition of DEX in culture medium improved relaxation to ACh and 10 μM of DEX totally restored the maximal relaxing response to ACh (30 ± 4 and 64 ± 3% for 1 and 10 μM).
Conclusion: These results show that, 7 days of culture develop a hyper-sensitivity of mice PA to contractile agonist which is prevented by DEX. Endothelium-dependent relaxation is largely reduced by the period of culture whereas relaxing capacity of smooth muscle cell was still preserved. DEX exerts a beneficial effect on culture-induced impairment of endothelium-dependent relaxation.

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Coronary endothelial dysfunction in mice with conditional, cardiomyocyte specific overexpression of the mineralocorticoid receptor
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Introduction: Deleterious effects of aldosterone excess have been demonstrated in cardiovascular diseases, and they might be linked in part to coronary vascular dysfunction. However, whether such vascular dysfunction is a cause or a consequence of the changes occurring in the cardiomyocytes is largely unclear. Moreover, the possible link between aldosterone-mediated effects on the cardiomyocyte on one hand and the coronary arteries on the other hand are unknown. Thus, we used a mouse model of conditional, cardiomyocyte-specific overexpression of MR (Ouvrard-Pascaud et al, Circulation 2005) and observed its effects on coronary endothelial function.
Methods: Three-months-old male transgenic mice (TG) overexpressing human MR in cardiomyocytes and their matched controls (Cont) were either untreated or treated with MR antagonist canrenoate (40 mg/kg/day for 1 month). Segments of left coronary arteries (diameter 180–220 μM) were isolated and mounted in a wire myograph. After pre-constriction with serotonin, we assessed endothelium-dependent, NO-dependent and independent relaxations in response to increasing concentrations of acetylcholine (Ach) before and after incubation with a NOSynthase inhibitor (LNNA) respectively.
Results: Compared to control mice, MR cardiac overexpression induced decreased relaxing responses to Ach either without (maximal relaxation: Cont: 95 ± 4% $n = 7$; TG: 68 ± 8% $n = 7$ $P < 0.05$) or with LNNA (Cont: 19 ± 3% $n = 7$; TG: 5 ± 4% $n = 7$ $P < 0.05$). A one-month treatment by canrenoate totally prevented this coronary dysfunction. The endothelium-independent relaxing responses to the NO-donor nitroprusside were similar in all groups.
Conclusion: We thus demonstrate that an increase in MR expression, restricted to cardiomyocytes is sufficient to induce a coronary endothelial dysfunction. This suggests for the first time that the phenotypic changes induced by aldosterone within the cardiomyocyte are sufficient to induce per se a secondary coronary vascular dysfunction.

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The aldosterone-induced coronary dysfunction in transgenic mice involves the BKCa channels of vascular smooth muscle cells
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Introduction: Cardiomyocyte specific overexpression of aldosterone-synthase in male (AS) mice induces NO-independent coronary dysfunction, but the mechanisms are unclear. Because large calcium-activated potassium (BKCa) channels are essential for vascular smooth muscle cell (VSMC) relaxation, we hypothesized that aldosterone might alter BKCa channel expression and/or function in VSMC.
Methods: Left coronary artery segments were isolated from male wild-type (WT) and AS 3-month-old mice either untreated or treated with the mineralocorticoid receptor antagonist spironolactone (20 mg/kg/day for 3 weeks) and mounted in a wire myograph. The acetylcholine-mediated coronary relaxation (in the presence of a NOSynthase inhibitor) was measured in the absence and in the presence of the BKCa inhibitor iberiotoxin, and the relaxing responses to the BKCa activator NS-1619 were also assessed. BKCa-α and -β1 subunits expression were quantified in mice hearts by RT-quantitative PCR and Western blot. Aldosterone and spironolactone effects on BKCa expression were studied in cultured rat aortic VSMC.
Results: Compared to WT mice, the acetylcholine-mediated coronary relaxation was markedly decreased in AS and this alteration was prevented by spironolactone. After iberiotoxin incubation, coronary relaxation to acetylcholine was markedly reduced and reached the same level in both AS and WT mice. AS coronary arteries were also less sensitive to NS-1619 than WT. Furthermore, BKCa-α and -β1 subunit expressions were decreased in AS hearts both at mRNA and protein levels. In cultured VSMC, aldosterone decreased BKCa channel expression at 24 h by 60% for α ($P = 0.001$) and 40% for β1 ($P = 0.001$) and spironolactone prevented these effects.
Conclusion: Aldosterone overexpression altered VSMC BKCa expression and coronary BKCa-dependent relaxation. The resulting alteration of EDHF-mediated response may contribute to the deleterious effect of aldosterone in cardiovascular diseases.

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Postocclusive reactive hyperemia inversely correlates with urinary 15-F2t-isoprostane levels in systemic sclerosis
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Introduction: Microvascular dysfunction and increased oxidative stress are major hallmarks of the systemic sclerosis disease process. The primary objective of this study was to test whether there is a link between peak postocclusive hyperemia and urinary levels of the F2-isoprostane 15-F2t-IsoP in patients suffering from systemic sclerosis.
Methods: We enrolled 43 patients suffering from systemic sclerosis, 33 patients with primary Raynaud 's phenomenon (RP), and 25 healthy volunteers. Microvascular function was assessed using the postocclusive hyperemia monitored by laser Doppler flowmetry. Endothelium-independent response was monitored after 0.4 mg sublingual nitroglycerin. Oxidative stress status was assessed by urinary levels of the F2-isoprostane 15-F2t-IsoP using GC–MS.
Results: The peak postocclusive vascular conductance was altered in subjects with systemic sclerosis and primary RP compared to controls (respectively 28 (7–48), 30 (13–48), and 39.9 (13–63) mV/mm Hg, $P = 0.01$). F2-isoprostanes were increased in the systemic sclerosis group compared to primary Raynaud's phenomenon and healthy controls (respectively 230 (155–387), 182 (101–284), and 207 (109–291) pg/mg, $P = 0.006$). In patients suffering from systemic sclerosis, there was a significant inverse correlation between F2-isoprostanes and postocclusive hyperemia, expressed as raw data ($R = -0.45$, $P = 0.007$) or as an increase over baseline ($R = -0.28$, $P = 0.04$). Conversely, no correlation was found with the nitroglycerin response.
Conclusion: In conclusion, we provide evidence that there is an inverse correlation between postocclusive hyperemia and urinary F2-isoprostane levels in patients suffering from systemic sclerosis. Whether oxygen free radicals initiate the vascular dysfunction or whether there is an initial trigger that initiates both processes will need to be further clarified in future studies.

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Exposure to urban air pollutants altered endothelial function in healthy subjects
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Introduction: Exposure to urban air pollution, ultrafines particles or gas, is associated with acute cardiovascular mortality and morbidity. We investigated the effect of ambient air pollution on endothelial function in 40 healthy Caucasian men, previously described in KLK study (JCI, 2005, 115 (3): 780–7), spontaneously breathed ambient air pollution in Paris.
Methods: Endothelial function was measured by the percentages of dilatation (dDr) and the absolute variation of blood velocity (dVel) and shear stress (dSS) after hyperhemia following 5 min hand ischemia and after 150 μg of TNT sublingual using RF-bases echotracking device, at two distinct visit 2 weeks apart. Air pollution level, (CO, NO, NO_2, SO_2, PM 2.5) were extracted from 'Airparif' database, the day of vascular measurement (J0'Pollutant') and 5 days before (mean 'pollutant'). The ranks of pollutants were added to form SPOL score.

Results: Mean pollutant levels were more closely correlated with endothelial parameters than J0 levels. Baseline dDr was significantly and negatively correlated with NO ($P = 0.0005$), SO2 ($P = 2.10^{-6}$) and JO SO2 ($P = 2.10^{-6}$), CO ($P = 7.10^{-5}$) and J0 CO ($P = 0.008$), SPOL ($P = 0.001$). SPOL explain 19% of the variance of baseline dDr. (1) Baseline dSS and dVel were significantly correlated with PM 2.5 ($P = 0.02$) and PM10 ($P = 0.001$). (2) Changes in dDr between the two visit was significantly correlated with delta NO ($P = 0.001$) and delta SPol ($P = 0.006$). Delta SPol explain 8% of the variance of Delta DdLir. (3) Changes in diameter or shear stress after TNT were not correlated with changes in air pollutants levels.
Conclusion: Endothelial function is significantly impaired by ordinary level of pollutant in healthy young males, in an urban area. Gazeous pollutants influence large artery endothelium whereas particulate matters influence small arteries response.

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Characterization of the endothelial β2-adrenoceptor-mediated signalling pathway in mice pulmonary arteries

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Introduction: We have previously shown that in mice pulmonary arteries, the β_2-adrenoceptor stimulation elicits a relaxation which is inhibited by a NO synthase (NOS) inhibitor or endothelium removal. The objectives of this study were (i) to assess the endothelial expression of the β_2-adrenoceptor in mice pulmonary arteries; (ii) to evaluate the role of different protein kinases (which are described to phosphorylate the endothelial NOS (eNOS) and regulate its activity) in the β_2-adrenoceptor-mediated relaxation; and (iii) to investigate the β_2-adrenoceptor-mediated response in mice with genetic deletion of eNOS ($eNOS^{-/-}$).
Methods: Extralobar pulmonary arteries were removed from male C57BL/6 wild-type or $eNOS^{-/-}$ mice (10–12 week-old). These vessels were mounted in a wire myograph and the effect of a selective β_2-adrenoceptor agonist (procaterol) was evaluated after submaximal precontraction with prostaglandin $F_{2\alpha}$. Immunohistochemistry experiments were also performed on mice pulmonary arteries sections using a polyclonal anti-β_2-adrenoceptor antibody.
Results: Immunohistochemistry experiments show a β_2-adrenoceptor staining at the endothelium layer of extralobar pulmonary arteries isolated from wild-type mice. In this arteries, procaterol induced a relaxation which was abolished in the presence of a NOS inhibitor (L-NAME, 300 μM), but not modified in the presence of the protein kinase A (PKA) inhibitor (Rp-8-Br-cAMPS, 100 μM), the phosphoinositide-3 kinase (PI3 K) inhibitors (LY294002, 10 μM; or wortmannin, 100 nM) or the extracellular signal-regulated kinase 1/2 (ERK1/2) inhibitor (PD98059, 20 μM). In pulmonary arteries isolated from $eNOS^{-/-}$ mice, the relaxation induced by procaterol was decreased but not abolished compared to that obtained in wild-type mice. This remaining relaxation elicited by procaterol in $eNOS^{-/-}$ pulmonary arteries was not modified by L-NAME, but significantly decreased in the presence of the combination of L-NAME, a cyclooxygenase inhibitor (indomethacin, 10 μM) and two K^+ channels blockers (apamin, 100 nM; and charybdotoxin, 100 nM).
Conclusion: These data demonstrate that the β_2-adrenoceptor is functionally expressed in endothelial cells of mice pulmonary arteries. They suggest that the β_2-adrenoceptor coupling to eNOS is independent of PKA, PI3K or ERK1/2 pathways. Furthermore, they show that genetic deletion of eNOS promotes a switching of the β_2-adrenoceptor coupling from eNOS to an EDHF- and/or cyclooxygenase-dependent relaxant pathway.

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Effects of cannabinoids on basal tone and on cholinergic-mediated contraction of human bronchi

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Introduction: The current study was performed to investigate whether cannabinoid (CB) receptor agonists (WIN55,212−2: CB1/CB2 agonist and JWH-133: CB2 agonist) could alter the basal tone of isolated human airways and also modulate the cholinergic contraction induced either by electrical field stimulation (EFS) or exogenously applied acetylcholine (ACh).
Methods: Macroscopically nondiseased human bronchial tissue was obtained from 43 patients with respiratory tumours. Responses induced by EFS or ACh were studied in bronchial rings mounted in organ baths containing modified Krebs–Henseleit solution containing indomethacin (10^{-6} M) and a CysLT1 receptor antagonist, MK476 (10^{-6} M). EFS (biphasic pulse width 1 ms, constant current of 320 mA for 10 s at 5 Hz) producing one third of the contraction induced by ACh (10^{-3} M) was monitored for 240 min. ACh-induced contraction was studied by cumulative addition of increasing concentrations of ACh (10^{-8} M to $3x10^{-3}$ M). Human bronchial rings were incubated with WIN55,212-2 or JWH-133 (10^{-7} à 10^{-5} M) for 30 min before EFS- or Ach-induced contractions.
Results: The cannabinoid agonists had no direct effect on basal tone and did not displace the concentration-response curve to Ach whereas the two compounds attenuated the EFS-induced contractions. Concentration-related inhibition of EFS-induced response was observed with WIN 55,212−2 and was significant at relatively low concentrations ($3x10^{-7}$ M). A significant attenuation was only observed at high concentrations of JWH-133 with dual agonist activity for the CB1/CB2 receptors. In addition, the inhibitory effect of WIN55,212-2 was reversed by a 1-hour pre-incubation with the CB1 receptor-selective antagonist (SR141716: 10^{-7} to 10^{-6} M) but not with the CB2 receptor-selective antagonist (SR144528: 10^{-5} M).
Conclusion: These results suggest that CB1-receptor stimulation inhibits the cholinergic contraction in human isolated bronchi through stimulation of prejunctional receptors, located to post-ganglionic cholinergic nerves.

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Carotid IMT and stiffness, aortic stiffness and pulse pressure: association with hormone therapy in postmenopausal women: baseline findings from the Cashmere trial

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Introduction: Common carotid artery intima media thickness (CCA-IMT), aortic stiffness (PWV) and central pulse pressure (PP) are early markers of atherosclerosis. The influence of hormonal replacement treatment (HRT) on arterial parameters in menopausal women remains to be investigated.
Methods: We used baseline data of 664 menopausal women with hypercholesterolemia, screened for the Cashmere study, a 12-month double-blind randomized trial comparing the effects of atorvastatin (80 mg/day) vs. placebo, ± HRT, on the progression of CCA-IMT. CCA-IMT, PP, PWV were measured by using a high-definition echotracking device (Esaot ®), aplanation tonometry (Sphygmocor®), and Complior® respectively.
Results: Mean age was 58 ± 6 years with a mean duration of menopause (M) of 8 ± 7 years. Age at M was 50 ± 5 years. 17% were smokers, 23% had hypertension and 28% were HRT users.

Independent determinant	CCA-IMT (μm)			Central PP (mmHg)			PWV (m/s)		
	b	R^2	*P*	b	R^2	*P*	b	R^2	*P*
Age at M (5 yrs)	25	2.9	<0.001	3.0	4.0	<0.001	0.4	4.7	<0.001
M. Duration (5 years)	25	4.8	<0.001	3.5	7.2	<0.001	0.6	12.4	<0.001
Current use of HT (yes)	−37	2.3	0.002	−2.7	0.9	0.003	−0.3	0.9	0.01
Mean BP (10 mmHg)		-	-	7.0	32.4	<0.001	0.4	8.8	<0.001
Central PP (10 mmHg)	9	1.3	0.004	-	-	-	-	-	-
Total R^2		13.2			48.3			24.0	

R^2 increment:% of explained variance, bcoef: slope of the multivariate correlation.

Conclusion: Duration and age at menopause were associated with thickening and stiffening of large arteries. Current users of HRT had significantly thinner and more distensible arteries than non users.

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Evidence for a role of NO synthase uncoupling in the alterations of vasomotor responses induced by chronic hypoxia in mice pulmonary arteries

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Introduction: Exacerbated vasoconstriction and endothelial dysfunction are key elements in the pathogenesis of pulmonary arterial hypertension induced by chronic hypoxia. In the present study, we investigate whether uncoupled NO synthase, which may produce reactive oxygen species following depletion of the NO synthase cofactor tetrahydrobiopterin, is involved in hypoxia-induced alterations of vasomotor responses (hyperreactivity to vasoconstrictors and endothelial dysfunction) in pulmonary arteries.
Methods: Male C57BL/6 mice (10–12 week-old) were exposed or not to hypobaric hypoxia (0.5 atm) for 21 days. Extrapulmonary arteries were removed for *in situ* staining of reactive oxygen species with the fluorescent dye dihydroethidium, and for assessment of contraction to the α_1-adrenoceptor agonist, phenylephrine, and of endothelial NO-dependent relaxation to the muscarinic agonist, acetylcholine, using a wire myograph.
Results: Compared to controls, pulmonary arteries from hypoxic mice displayed an increase in dihydroethidium staining (which was blunted by the permeant scavengers of reactive oxygen species, polyethyleneglycol-superoxide dismutase or polyethyleneglycol-catalase), an increase in phenylephrine-induced contraction, and a decrease in acetylcholine-induced relaxation. In pulmonary arteries from hypoxic mice (but not in those from controls), contractile effect of phenylephrine was diminished in the presence of polyethyleneglycol-superoxide dismutase, catalase or sepiapterin (a precursor of the NO synthase cofactor, tetrahydrobiopterin). However, tetrahydroneopterin (a tetrahydrobiopterin analog, which is not a cofactor of NO synthase) failed to modify phenylephrine-induced contraction in pulmonary arteries from hypoxic mice. Chronic oral treatment of mice with sepiapterin during exposure to hypoxia not only attenuated hyperreactivity to phenylephrine, but also fully restored the endothelial NO-dependent relaxation to acetylcholine.
Conclusion: These data show that in pulmonary artery from hypoxic mice, elevation of reactive oxygen species mediates hyper responsiveness to contractile agents. The beneficial effects of sepiapterin on hypoxia-induced alterations of vasomotor responses (i.e. decrease in hyperreactivity to vasoconstrictors and improvement of endothelial function) support the idea that uncoupled NO synthase plays a key role in the pathogenesis of pulmonary arterial hypertension induced by chronic hypoxia.
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Hormone therapy and risk of venous thromboembolism among postmenopausal women. Influence of the Cytochrome P450 1A2 genetic polymorphism (CYP1A2*1F)

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Introduction: Oral estrogen replacement therapy increases the risk of venous tromboembolism (VTE). Transdermal estrogen may be safe with respect to thrombotic risk.

The cytochrome P450 1A2 (CYP1A2) is partly responsible for the metabolism of estrogens and many exogenous compounds including caffeine. A single nucleotide polymorphism (SNP) in intron 1 (−163C → A) of the *CYP1A2* gene (*CYP1A2**1F) influences the extent to which CYP1A2 induced in cigarette smokers. *CYP1A2**1F allele is associated with increased CYP1A2 inducibility in Occidentals and Chinese smokers but not in non-smokers.
In this case–control study, we investigated the clinical significance of *CYP1A2**1F polymorphism on the association between smoking habits, hormone therapy by route of estrogen administration and VTE risk.
Methods: We reanalyzed the data from a multicenter case–control study of VTE among postmenopausal women who were enrolled in 1999 through 2006 at eight clinical centers and in the general population, in France. The *CYP1A2* genetic polymorphism (allele *CYP1A2**1F), was successfully evaluated in 193 consecutive cases with a first documented episode of idiopathic VTE and in 530 controls. Relative risks were estimated by odds ratios (OR) and 95% confidence intervals.
Results: The *CYP1A2**1F allele frequency was 72% and 71% among cases and controls, respectively (*OR* = 1.0; 95% CI: 0.7–1.4). Oral but not transdermal estrogen increased VTE risk compared with non-users (*OR* = 3.8; 95% CI: 2.3–6.5 and *OR* = 1.2; 95% CI: 0.8–1.8 respectively) in all patients carriers of *CYP1A2**1F allele. Compared with non-users, OR in current users of oral estrogen was 1.6 (95% CI: 0.4–6.7) among smokers carriers of *CYP1A2**1F allele and 6.6 (95% CI: 0.5–84.7) among non-smokers.
Conclusion: Among women using oral estrogen substitution and with smoking habit, carriers of *CYP1A2**1F allele seem to present a lower risk of VTE. If this result can be confirmed, it may be explained by a higher estrogen clearance among *CYP1A2**1F allele carriers.

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Chronic diet with red wine polyphenols alters NO-dependent reactivity in pulmonary arteries from normoxic and hypoxic mice
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Introduction: Red wine polyphenols exert various vascular effects, some of them likely contributing to the benefits of moderate red wine intake on cardiovascular diseases, which are reported in epidemiological studies. In this study, we determined the effects of chronic treatment with red wine polyphenols on NO-dependent reactivity in mice pulmonary arteries.
Methods: Male C57BL6 mice (11–13 week-old) were exposed or not to hypobaric hypoxia (0.5 atm for 21 days) and treated with a red wine polyphenolic extract (100 mg/kg by oral administration, 3 days a week for 21 days) or vehicle (10% ethanol, pH 3.3). Extra- and intra-lobar pulmonary arteries were excised and mounted in a wire myograph.
Results: In pulmonary arteries from normoxic mice, the NO-synthase inhibitor N^{ω}-nitro-L-arginine methylester (L-NAME) potentiated contraction to $PGF_{2\alpha}$ and abolished endothelium-dependent relaxation to acetylcholine. Treatment of normoxic mice with red wine polyphenols did not modify the maximum contraction induced by $PGF_{2\alpha}$ (obtained either in the absence or presence of L-NAME), while it enhanced relaxation to acetylcholine in intra- but not extra-pulmonary segments. In pulmonary arteries from hypoxic mice, contraction to $PGF_{2\alpha}$ was markedly enhanced, L-NAME failed to potentiate $PGF_{2\alpha}$-induced responses and relaxation to acetylcholine was markedly diminished. Treatment of hypoxic mice with red wine polyphenols restored the potentiating effect of L-NAME on $PGF_{2\alpha}$-induced contraction in extra-pulmonary arteries and decreased the contraction to $PGF_{2\alpha}$ towards control levels in intra-pulmonary segments. In both extra- and intra-pulmonary arteries from hypoxic mice, the red wine polyphenols treatment did not modify the residual relaxation to acetylcholine.
Conclusion: These data show that intake of red wine polyphenols exerts different beneficial effects on pulmonary arteries, depending on housing conditions (normoxia/chronic hypoxia) and arterial segments (extra-/intra-pulmonary arteries). The amplification or restoration of NO relaxant influence induced by red wine polyphenols likely exerts protective effects in the pulmonary vasculature.
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Cerebral vascular remodeling in apolipoprotein E knockout mice with chronic renal failure
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Introduction: Little is known about the impact of chronic renal failure (CRF) on cerebral circulation, a surprising situation considering the high risk of ischemic and hemorrhagic stroke in this population. We examined structure and function of cerebral arterioles in a well defined model of apolipoprotein E knockout ($apoE^{-/-}$) mice with CRF.
Methods: We measured systemic mean arterial pressure (MAP, mmHg) and cerebral arteriolar internal diameter (ID, μM) in anesthetized $apoE^{-/-}$ and C57BL/6 J mice (WT) with and without CRF (induced by electrocauterisation of the right kidney followed by controlateral left nephrectomy). We examined autoregulation-induced vasodilatation (AIV, μM) by measuring ID prior to and during stepwise hypotension (hemorrhage, 10 mmHg per steps). ID was also measured during a second stepwise hypotension after complete deactivation of cerebral arterioles with EDTA (67 mmol/L).
Results: Results are expressed as mean ± SEM (**P* = 0.05 vs. WT, $^{\dagger}P$ = 0.05 vs. sham, two ways ANOVA). ID_{EDTA} was measured at a MAP of 30–40 mmHg.

	WT (*n* = 11)	CRF WT (*n* = 11)	$apoE^{-/-}$ (*n* = 9)	CRF $apoE^{-/-}$ (*n* = 12)
MAP	59 ± 3	52 ± 3	63 ± 3	60 ± 2
Baseline ID	38 ± 1	39 ± 2	48 ± 2*	47 ± 2*
AID	5 ± 1	1 ± 1†	6 ± 1	4 ± 2†
ID_{EDTA}	49 ± 2	44 ± 2†	59 ± 2*	53 ± 2*†

PAM and baseline ID were not modified by CRF. In contrast, ID measured after deactivation with EDTA was significantly reduced by CRF in both WT and $apoE^{-/-}$ mice. Finally, AIV was impaired in WT and $apoE^{-/-}$ mice with CRF.
Conclusion: Our results suggest that cerebral arterioles might undergo vascular remodeling during CRF, which might impair AIV.

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Pressure-induced myogenic tone is reduced in pulmonary artery of chronic hypoxic mice
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Introduction: Chronic hypoxia (CH) induces increase in pulmonary vascular resistance (PVR), and leading to pulmonary arterial hypertension. A potential contributor to elevated PVR is stretch-induced constriction or myogenic tone that may enhance vasoconstrictor reactivity following CH. To test this hypothesis, pressure-induced vasoconstriction has been evaluated in isolated pulmonary arteries (PA) from mice exposed or not to CH. The role of endothelium and nitric oxide (NO) was also characterized in mice pulmonary arteries from both groups.
Methods: Male C57BL6/J mice were exposed or not to hypobaric hypoxia (0.5 atm) for 21 days. Small extralobar segments (internal diameter <400 μM) were cannulated at one or both ends using an arteriograph system and subjected to pressure increments with simultaneous measurements of internal diameter. Diameter-pressure curves were constructed in the presence (2 mM) and absence of calcium to determine the contractile response. This pressure-dependent contractile response or myogenic tone has been expressed as% of contraction induced by high potassium solution (80 mM K^{+}).
Results: Over the whole range of pressure tested (5–50 mmHg), a myogenic tone was observed in PA from control and CH mice. However, the amplitude of the response was smaller in PA from CH mice compared to control. At the level of operating pressure present *in situ* (15 and 30 mmHg in PA from control and CH mice, respectively), a 30% reduction in the contractile response was observed in PA from CH mice (15 ± 2 vs. 24 ± 3%, $P = 0.05$ in CH and control, respectively). The NO synthase inhibitor, N^{ω}-nitro-L-arginine methymester (L-NAME), at a concentration (300 μM) which totally abolished the endothelium-dependent relaxant effect of acetylcholine, did not modify the myogenic response developed in PA from control and CH mice. No change of the myogenic response was also obtained after endothelium removal in PA from both groups of mice.
Conclusion: These results show that, myogenic tone is present in mice PA. Furthermore, this pressure-dependent contractile response is NO- and endothelium-independent. Finally, as this myogenic response is decreased following CH, elevation of PVR cannot be attributed to a rise in myogenic tone in this PA.

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Proportion of the antihypertensive treatment effect on the relative reduction of cardio-vascular risk explained by blood pressure
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Introduction: The objective of this study was to assess the part of the antihypertensive treatments effect on cardio-vascular events explained by the blood pressure and how this part is changing over time.
Methods: The proportion of treatment effect explained by an intermediate criterion (systolic blood pressure) was computed by comparing directly the unadjusted relative reduction of cardio-vascular risk due to treatment (overall treatment effect) with the relative reduction of risk due to treatment adjusted on the intermediate criterion (part of the treatment effect which is independent of the systolic blood pressure). To take into account the evolution over time, the relative reductions of risk were computed from survival models.
Results: The method was applied to the data of the SHEP study, which is a randomized, placebo-controlled, double-blind clinical trial, to test the effect of diuretics and beta-blockers on coronary heart disease event and stroke. The relative risk reduction of coronary heart disease events has been shown important the first year of follow-up, but decreasing thereafter until the fifth year. Both relative reductions of risk, unadjusted and adjusted on the systolic blood pressure, were close, and the proportion of treatment effect explained by the systolic blood pressure was close to zero during all the follow-up. For stroke, the overall treatment effect was beneficial during the entire trial period, whereas the part of the treatment effect independent of systolic blood pressure was harmful during the first 2.5 years, to become protective thereafter. Thus, the proportion of treatment effect explained by systolic blood pressure was above 100% until 2.5 years and then decreased until 70%.
Conclusion: We propose a new expression of the treatment effect separating the parts attributable to and independent of the intermediate criterion. The application of this expression to the SHEP study suggests that all the antihypertensive treatment effect on coronary heart disease events is explained by other mechanisms than their effect on lowering the systolic blood pressure. Their effect on stroke is only partly explained by their effect on lowering the systolic blood pressure, in a proportion which varies along time.

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Cross-link between PI3-kinase and MAP kinase pathways in the regulation of NO signaling and increase in reactive oxygen species production by apoptotic T lymphocyte microparticles in endothelial cells
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Introduction: Microparticles (MPs) are membrane vesicles with procoagulant and proinflammatory properties released during cell activation. We have previously shown that the MPs from T cells induce endothelial dysfunction by alteration of NO and prostacyclin pathways, but the mechanisms implicated are not elucidated yet. The present study was designed to dissect the signaling pathways of these MPs in endothelial cells with respect to both NO pathway and reactive oxygen species (ROS).

Methods: MPs were produced by treatment of human lymphoid CEM T cell line with the apoptotic agent, actinomycin D. Eahy 926 endothelial cells were grown for 24 h in absence or presence of 10 μg/mL microparticles pre-incubated or not either with PI3-kinase inhibitor (LY294002, 20 μM), MEK 1/2 inhbibtor (U0126, 10 μM),. Cell lysates were analyzed by Western blot. Also, cells were used for direct measurement of nitric oxide, whereas oxidative stress was determined by flow cytometry. Statistical analyses were performed by a one way analysis of variance (ANOVA), and Mann–Whitney U tests. $P = 0.05$ was considered to be statistically significant ($n = 5–6$).
Results: Incubation of Eahy 926 endothelial cell line with 10 μg/mL MPs for 24 h resulted in overexpression of endothelial NO synthase (eNOS) (150 ± 12%) and its phosphorylation on both activation and inhibition sites (Ser1177 and Thr495, 40 ± 7, 298 ± 17% respectively). Also, MPs enhanced expression of caveolin-1 (93 ± 4%) and decreased its phosphorylation on Tyr14 (75 ± 9%). Besides, MPs enhanced ROS production (23% of increase) measured with the fluorescent probe dihydroethidium (DHE) and decrease NO production (43%) measured with electron paramagnetic resonance technique. The inhibitor of the PI3-kinase, LY294002, reduced the effects of MPs on eNOS but not on caveolin pathways whereas it potentiated the effects of MPs on ROS production. In another set of experiments, MPs stimulated the ERK1/2 phosphorylation (73 ± 7%). As expected the MEK inhibitor, U0126, prevented ERK1/2 phosphorylation (48 ± 3%). Interestingly, U0126 reversed eNOS phosphorylation (Ser117 and Thr495 60 ± 5, 85 ± 3% respectively) but it have no effect on ROS production induced by MPs.
Conclusion: In summary, MPs activate multiple pathways related to NO and ROS productions through PI3-kinase. Beside, PI3-kinase controls the activation of ERK1/2 cascade which counteracts the increase of ROS production by the former. Altogether, these data underscore the pleiotropic effects of MPs on NO and ROS in endothelial cells leading to an increase oxidative stress in the vessel wall. The latter may account for the deleterious effects of MPs resulting in endothelial dysfunction.

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5-lipoxygenase pathway: one of the mediators of inflammation in obstructive sleep apnea syndrome?

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Introduction: Obstructive sleep apnea syndrome (OSAS) is associated with cardiovascular morbidity. Leukotrienes (LTs) are 5-lipoxygenase-derived metabolites involved in the pathogenesis of cardiovascular diseases. Our objectives were to evaluate in OSAS patients free of cardiovascular history and in healthy volunteers 1/the production of LTB_4 by polymorphonuclear leucocytes stimulated either by A23187 or arachidonic acid, 2/the urinary excretion of LTE_4, and 3/ the relationship between OSAS severity and LT production.
Methods: We prospectively studied 56 OSAS patients and 16 control subjects. LTB_4 and LTE_4 were quantified by liquid chromatography tandem mass spectrometry.
Results: LTB_4 production in response to A23187 (10 μM) or arachidonic acid (30 μM) was significantly increased in OSAS patients compared to controls. Moreover, the production of LTB_4 in response to A23187 or arachidonic acid was correlated with the mean nocturnal oxygen desaturation (SaO_2) ($R = –0.56$, $P = 0.001$). LTE_4 excretion was also higher ($P = 0.005$) in OSAS patients. In multivariate analysis, LTB_4 levels were associated with SaO_2 independently of confounding factors such as age or body mass index (BMI) whereas LTE_4 levels were correlated to BMI.
Conclusion: The activation of the two arms of the 5-lipoxygenase pathway may be an important new molecular mechanism in the pathogenesis of cardiovascular diseases in OSAS. Targeting 5-LOX pathway could represent a new therapeutic strategy to prevent the onset of cardiovascular disease in moderate to severe OSAS patients.

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Role of cyclooxygenases (COX-1 and COX-2), but not thromboxane A2, in hyperreactivity to vasoconstrictors induced by chronic hypoxia in mice pulmonary arteries

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Introduction: Exacerbated vasoconstriction plays a key role in chronic hypoxia-induced elevation of pulmonary arterial resistance. Our recent findings indicate that hyperresponsiveness of pulmonary artery to contractile agonists is mediated by reactive oxygen species produced by uncoupled NO synthase. As there exists close interplays between reactive oxygen species and cyclooxygenase pathways, this study investigates the potential contribution of cyclooxygenase-derived contractile prostanoids in hypoxia-induced hyperreactivity of pulmonary artery to vasoconstrictors.
Methods: Male C57BL/6 mice (10–12 week-old) were exposed or not to hypobaric hypoxia (0.5 atm) for 21 days. Extrapulmonary arteries were removed and mounted in a wire myograph for evaluation of contractile responses to receptor-dependent (phenylephrine, an $\langle_1$-adrenoceptor agonist) and receptor-independent (depolarising KCl) agents.
Results: Contractions induced by KCl and phenylephrine were markedly enhanced in pulmonary arteries from hypoxic mice, compared to controls (1.5–1.7 fold increase in maximal effect). In pulmonary arteries from hypoxic mice, contractile effect of phenylephrine was significantly diminished in presence of the selective cyclooxygenase-1 inhibitor (SC560, 1 μM), selective cyclooxygenase-2 inhibitor (NS398, 1 μM), and thromboxane A_2 receptor (TP) antagonists (SQ29548, 0.5 μM; or L670596, 1 μM). However, in these arteries, the thromboxane A_2-synthase inhibitor (furegrelate, 10 μM or 100 μM) did not modify significantly phenylephrine-induced contraction. Catalase (250 U/ml), which decomposes hydrogen peroxide, also decreased phenylephrine-induced contraction in pulmonary arteries from hypoxic mice. Combination of catalase and L670596 did not induce greater inhibition of contraction than catalase or L670596 alone in these arteries. None of these agents affected the contractile effect of phenylephrine in pulmonary arteries from control mice.
Conclusion: These data provide evidence that, in mice pulmonary arteries, hypoxia-induced hyperresponsiveness to contractile agents is mediated by a common pathway involving both hydrogen peroxide and cyclooxygenases, including the inducible cyclooxygenase-2. In these arteries, activation of TP receptors by another mediator than thromboxane A_2 is likely responsible for exacerbated vasoconstriction.
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Digital thermal hyperaemia impairment does not realte to skin fibrosis or macrovascular disease in systemic sclerosis

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Introduction: Thermal hyperemia is impaired in patients with systemic sclerosis (SSc). The objective of these studies was to determine whether this was consecutive to skin fibrosis, microangiopathy or a macroangiopathy.
Methods: Using laser Doppler flowmetry, we compared the thermal hyperemia on the third left finger pad and on the left forearm in 21 patients with non-diffuse systemic sclerosis, in comparison with primary Raynaud's phenomenon and healthy volunteers. We secondly tested whether the altered thermal hyperemia correlated to the digital pressure index at baseline and following the thermal challenge.
Results: In the first study, thermal hyperemia of the finger pad was impaired in terms of both amplitude and kinetics, but not on the forearm in patients with SSc. In the seven SSc patients without cutaneous fibrosis, the response was similarly altered in terms of amplitude and kinetics. In the second study, we observed a weak correlation between the digital systolic blood pressure index and the 44°C thermal plateau. However, in the 15 SSc patients tested at 44°C, the median digital systolic blood pressure index was 1.04 (0.84–1.24) at baseline vs. 1.08 (0.87–1.29) at 44°C (NS), while seven of them had an abnormal response in terms of kinetic. Furthermore, only one patient showed a clear-cut decrease in digital systolic blood pressure at 44°C.
Conclusion: In patients with SSc, digital thermal hyperemia is impaired, but does not relate to the skin fibrosis or to an associated macroangiopathy in most cases. Further studies are required to determine whether its impairment reflects a functional or structural microvascular damage.

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Crucial role of NO and EDHF in human sustained conduit artery flow-mediated dilatation

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Introduction: Whether nitric oxide (NO) is involved or not sustained conduit artery flow-mediated dilatation in humans remains unclear. Moreover, the role of endothelium-derived hyperpolarizing factor (EDHF), synthesized by cytochrome epoxygenases and acting through calcium-activated potassium channels, and its relationship with NO during flow-mediated dilatation have never been previously investigated.
Methods: In twelve healthy subjects we measured radial artery diameter (echotracking) and blood flow (Doppler) during flow-mediated dilatation induced by gradual hand skin heating (34–44°C), during the local infusion of saline and inhibitors of NO-synthase (L-NMMA: 8–20 μmol/min/L), calcium-activated potassium channels (tetraethylammonium chloride: 9 μmol/min/L), and cytochrome epoxygenases (fluconazole: 0.4–1.6 μmol/min/L), alone and in combination. Mean wall shear stress, the flow-mediated dilatation stimulus, was calculated at each level of flow and diameter-wall shear stress relationship was constructed. Furthermore, reactive oxygen species were quantified in local blood samples by electron paramagnetic resonance spectroscopy in six additional healthy volunteers during a control hand skin heating.
Results: During heating, the increase in blood flow was unaffected by tetraethylammonium while it was reduced, in the same extent, by L-NMMA alone and in combination with fluconazole and with tetraethylammonium (all $P = 0.05$). In contrast, hyperemia was enhanced by fluconazole ($P = 0.05$). Moreover, heating was associated with an increase in reactive oxygen species from 29.6 ± 2.9 to 38.3 ± 4.1 μmol/L ($P = 0.05$). Concerning the conduit artery, compared with saline, the diameter-shear stress relationship was shifted downwards by L-NMMA, tetraethylammonium, fluconazole, and in a more pronounced manner, by the combinations of L-NMMA with tetraethylammonium or with fluconazole (all $P = 0.05$). Therefore, the maximal radial artery flow-mediated dilatation, compared with saline (0.62 ± 0.03 mm), was decreased under our experimental conditions by L-NMMA (-39 ± 4%, $P = 0.05$), tetraethylammonium (-14 ± 4%, $P = 0.05$), fluconazole (-18 ± 6%, $P = 0.05$), and to a greater extent, by the combinations of L-NMMA with tetraethylammonium (-64 ± 4%, $P = 0.05$) or with fluconazole (-71 ± 3%, $P = 0.05$).
Conclusion: This study demonstrates that NO and a cytochrome-related EDHF play a crucial role *in vivo* in human peripheral conduit artery flow-mediated dilatation during sustained flow conditions and strongly suggests a functional interaction between both pathways to maintain this endothelium-dependent vasomotor response. Furthermore, the blood flow data suggest that a cytochrome-related vasoconstrictor mechanism, probably related to the release of reactive oxygen species, regulate in balance with NO the skin arteriolar dilatation to heating.

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Characterization of endothelin receptors in the isolated perfused mouse kidney

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Introduction: Endothelin-1 (ET-1) is a potent renal vasoconstrictor peptide that could be implicated in acute renal failure. ET-1 causes contractions through ET_A and ET_B receptors, but may also induce relaxations through ET_B receptors. The present study aimed to characterize the responses to ET-1 in isolated perfused mouse kidneys by using BQ-123 (selective ET_A receptor antagonist) and BQ-788 (selective ET_B receptor antagonist).

Methods: The mouse kidneys were perfused with oxygenated Tyrode solution at 37°C and changes in renal perfusion pressure were recorded and taken as an index of renovascular resistance changes. The substances were injected as a bolus of 5 μL (dose expressed in mole) or infused (concentration expressed in μM).
Results: Increasing doses of ET-1 and sarafotoxin 6c (ET_B–selective agonist) caused potent vasoconstrictions with a maximal amplitude of 183 ± 7 and 116 ± 13 mmHg and an EC_{50} of 6.2 and 2.6 pmol, respectively. At the dose of 6 pmol, the constrictions to ET-1 showed a long duration of action (>30 min) while those to sarafotoxin 6c (10 pmol) were transient. ET-1-induced constrictions were partially inhibited by BQ-123 (0.1 and 1 μM) or by BQ-788 (0.1 and 1 μM) but completely blocked by the combination of the two antagonists. In the presence of the both antagonists, the constrictions to noradrenaline and angiotensin II were not changed. Sarafotoxin 6c-induced constrictions were not affected by BQ-123, but selectively blocked and in a dose-dependent manner by BQ-788. The maximal constrictor responses to ET-1 were not affected by a NO-synthase inhibitor, L-nitro-Arginine (100 μM).
Conclusion: These findings show that in the isolated perfused mouse kidney, ET-1 is a potent vasoconstrictor agent that induces constrictions by stimulating both ET_A and ET_B receptors present at the level of the vascular smooth muscle. The data also suggest that stimulation of ET_A receptors is responsible for the long duration of the ET-1 response, while the activation of the both receptors is responsible for the initial phasic constriction. The ET_B receptors of endothelial cells mediating dilatory responses are not involved in this model. This study illustrates the important contribution of both ET_A and ET_B receptors in the renal resistance vessels in the mouse.

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Pulse wave velocity as independent predictor of subclinical atherosclerosis burden

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Introduction: Pulse wave velocity (PWV), the sclerotic component of atherosclerosis, is closely dependent of blood pressure (BP) and age, but it has been suggested that its elevation provide incremental prognostic information as compared to traditional risk factors.
Objective: To confirm the independent predictive value of PWV by testing its cross-sectional associations with risk factors and subclinical atherosclerosis taken as markers of global cardiovascular risk.
Methods: The study was performed with a hospital based cohort of 686 men and 279 women (51 ± 11 years) at increased risk for cardiovascular diseases, underwent risk factor assessment including ultrasonic detection of plaque at carotid, aorta and femoral sites and intima-media thickness (IMT) measurement. Mean IMT was deduced from both sides of common carotid artery. Carotid-femoral PWV was measured mecanographically with an automatic detection. Plaque burden was assessed by measuring the number of sites (0–5) with at least one present plaque among both carotid arteries, both femoral arteries, and the whole abdominal aorta.
Results: Results of PWV regression are shown in the table

Variables	Univariate *r*, *P*	Multivariate beta estimate, *P*
Sex	0.10, $P < 0.01$	0.11, NS
Age	0.46, $P < 0.0001$	0.07, $P < 0.0001$
Body mass index	0.17, $P < 0.0001$	0.04, $P < 0.01$
Systolic BP	0.51, $P < 0.0001$	0.06, $P < 0.0001$
IMT	0.34, $P < 0.0001$	1.45, $P < 0.01$
Plaque burden	0.30, $P < 0.0001$	0.09, $P < 0.02$

Conclusion: PWV is associated independently to risk factors, IMT and plaque burden, so supporting its additive value for reflecting global cardiovascular risk.

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C-type natriuretic peptide (CNP) is not an EDHF in the guinea-pig carotid artery

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Introduction: It has been recently suggested that, in the rat mesenteric artery, endothelium-derived CNP was an EDHF, which by stimulating smooth muscle NPR-C receptor subtype would hyperpolarize the smooth muscle cell by activating the G-protein regulated inward-rectifier K^+ channel (GIRK; 1). This study was designed to determine whether or not endothelium-dependent hyperpolarizations evoked by acetylcholine in the isolated guinea-pig carotid artery could also involve CNP.
Methods: The membrane potential of vascular smooth muscle cells was recorded in isolated carotid artery strips with intracellular microelectrode.
Results: In the presence of inhibitors of NO-synthases and cyclooxygenases, acetylcholine induced the endothelium-dependent hyperpolarization of the vascular smooth muscle cells, a phenomenon which was abolished by the combination of TRAM-34, a blocker of intermediate conductance calcium-activated potassium channel (IK_{Ca}) and apamin, a blocker of small conductance calcium-activated potassium channel (SK_{Ca}), but was unaffected by glibenclamide, a blocker of ATP-sensitive potassium channel (K_{ATP}). Atrial natriuretic factor (ANF) and CNP repolarized phenylephrine-depolarized arteries and hyperpolarized quiescent arteries. However, under both conditions, the changes in membrane potential elicited by ANF and CNP were significantly smaller than those produced by acetylcholine. Furthermore, CNP-induced hyperpolarizations were abolished by glibenclamide.
Conclusion: These results indicate that, in the guinea-pig isolated carotid artery, the release of CNP cannot account for the EDHF-mediated responses evoked by acetylcholine.

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Sonic hedgehog carried by microparticles corrects endothelial injury and promotes angiogenesis through nitric oxide release

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Introduction: Microparticles are small fragments generated from the plasma membrane after cell stimulation. Among the candidate proteins harboured by microparticles, we have recently shown that Sonic Hedgehog is present in MPs generated from activated/apoptotic human T CEM lymphocytes.
Methods: Microparticles were isolated following serial centrifugations. Eahy 926 endothelial cells were grown for 24 h in absence or presence of 10 μg/mL microparticles pre-incubated or not either with PI3-kinase inhibitor (LY294002, 20 μM), MEK 1/2 inhbibtor (U0126, 10 μM), cyclopamine (30 μM), a specific antagonist of the Sonic Hedgehog receptor (Patched), or siRNA of Patched. Cell lysates were analyzed by Western blot. Also, cells were used for direct measure of nitric oxide (NO), whereas reactive oxygen species (ROS) was determined by flow cytometry. In another set of experiments, after 24 h of *i.v.* of microparticles into mice, endothelium-dependent relaxation was determined in aortic rings. Also, ischemia/reperfusion was induced in mice by ligating the left anterior descending coronary artery proximal to its origin and endothelial function of distal coronary artery was assessed. Finally, the effect of microparticles on angiogenesis was determined by Matrigel assays and vessel sprouting in mice aortic rings. Statistical analysis were performed by a one way analysis of variance (ANOVA), and Mann–Whitney U tests or tow way ANOVA for repeated measures and subsequent Bonferroni post hoc test. $P = 0.05$ was considered to be statistically significant ($n = 4$–6).
Results: Here, we show that Sonic Hedgehog carried by microparticles induces NO release from endothelial cells at the basal level and after a bradykinin-stimulation (20 μM) (2.47- and 2.6-fold, respectively) and triggers changes in both the expression and the phosphorylation of enzymes related to the NO pathway, and also decreases production of ROS (38.6 ± 1.4% of positive cells in treated vs. 51.4 ± 0.2% in control). When PI3-kinase and ERK signalling were specifically inhibited, the effects of microparticles were reversed. *In vivo* injection of microparticles in mice was also able to improve endothelial function (Emax; 51.5 ± 2% vs. 71.5 ± 1.7% in control and microparticles-treated mice respectively, $P = 0.001$) by increasing NO release (1.4, 1.92 and 2.6-fold in blood, hearth and lung, respectively) and it reversed endothelial dysfunction after ischaemia/reperfusion. Silencing the effects of Sonic Hedgehog with either cyclopamine or siRNA of Patched strongly reduced the production of NO elicited by microparticles (34.6%). Finally, microparticles promote angiogenesis, these effects being abolished by Sonic Hedgehog pathway inhibition.
Conclusion: Taken together, we propose that the biological message carried by microparticles harbouring Sonic Hedgehog may represent a new therapeutic approach against endothelial dysfunction during acute severe endothelial injury.

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T lymphocyte-derived microparticles from Crohn's disease patients with active disease induce vascular hyporeactivity through a PPAR-gamma-dependent pathway

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Introduction: Several anatomical, pathological and physiological [1, 2] studies suggest that vascular reactivity is impaired in Crohn's disease (CD) patients, and may contribute to its pathophysiology. In addition, circulating cell-derived microparticles have been reported to be increased in active CD whereas returning to normal values in infliximab (a monoclonal anti-TNF-alpha antibody) treated patients.
Considering these data, the main objective of the presented study was to investigate the potential effects of T lymphocyte-derived microparticles (TLMP) from Crohn's disease (CD) patients with active disease, compared to healthy controls (HC) on vascular reactivity.
Methods: Circulating TLMP were obtained from peripheral venous blood in eight CD patients with active disease [Crohn's Disease Activity Index (CDAI) <220] and six HC. Their effects on vascular reactivity of male Swiss aortic rings (with endothelium or after its removal) mounted on a wired myograph was studied in presence or not of 30 nmol/L of TLMP from active CD patients or HC, after application of acethylcholine or serotonine, with or without rosiglitazone, a PPAR-gamma agonist.
Results: (1) Compared to TLMP from HC, TLMP from active CD patients induce a significant vascular hyporeactivity of acethylcholine-activated aortic rings ($P = 0.001$). (2) This is also observed after activation by serotonine in both intact aortic rings ($P = 0.001$) or after endothelium removal ($P = 0.01$). (3) These effects are associated to an increase in arterial cyclooxygenase 2 (COX2), but not nitric oxide, expression (as assessed by immunohistochemistry). (4) Rosiglitazone abolishes in a dose-dependent manner TLMP effects on vascular reactivity.
Conclusion: T lymphocyte-derived microparticles from patients with active CD induce a significant vascular hyporeactivity suggested to participate in CD pathophysiology, suggesting an important deleterious role of inflammation-induced TLMP in active CD. In this condition, increased COX2 expression in presence of TLMP can be interpreted as a defence mechanism which may protect from vascular hyporeactivity and its consequences in CD. Improvement of endothelial and vascular hyporeactivity by rosiglitazone indicates, in addition to other data on PPAR-gamma agonists in CD, that PPAR-gamma should be considered as a potential therapeutic target in CD.
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Chronic intermittent hypoxia in mice induces hyperlipidemia, inflammatory response and leukocyte rolling

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Introduction: Obstructive sleep apnea syndrome is characterized by repetitive intermittent hypoxia/reoxygenation, produced by recurrent collapses of the upper airway. This syndrome is correlated to an increase in cardiovascular morbidity, with a high risk of hypertension, coronary artery disease, arrhythmias and cerebrovascular events. The aim of the present study was to characterize the inflammatory response of mice chronically submitted to intermittent hypoxia/reoxygenation.

Methods and Results: C57BL/6 J mice were exposed to intermittent hypoxia or normoxia for 5, 14 or 35 days. Serum levels of total cholesterol were increased after 5 and 14 days of intermittent hypoxia, whereas at 35 days, these levels were comparable to that of mice submitted to normoxia. Splenocytes isolated from the hypoxic mice showed increased proliferation capacity. mRNA analysis, realized on spleen tissue of these mice, revealed an increased expression of the chemokines MCP-1, RANTES and MIP-1α in intermittent hypoxia conditions. Leukocyte rolling, measured *in vivo*, was significantly increased in mice exposed to intermittent hypoxia for 35 days, compared to mice submitted to normoxia.
Conclusion: These data demonstrate an inflammatory response in mice exposed to chronic intermittent hypoxia, which may better explain some of the cardiovascular complications of the obstructive sleep apnea syndrome.

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Effects of local anaesthesia on subdermal needle insertion pain and subsequent tests of microvascular function in human

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Introduction: Post occlusive and local thermal hyperemia are currently used as integrated tests to study microvascular function in microvascular diseases. However, further pathophysiological insight would require its association with microdialysis. The major limitation remains the microinvasive approach as local anaesthesia prior to fiber insertion could lead to confounding effects. The objective of our study was to determine whether EMLA® cream treatment (lidocaine prilocaine), applied for 20 min, 40 or 60 min, significantly decreases the pain related to intradermal needle insertions, while not decreasing the microvascular response to postocclusive reactive hyperemia (PORH) and thermal hyperemia 2 h after cream removal.
Methods: This was an open labelled parallel randomized controlled study, where each of the six subjects enrolled was its own control. Four sites were chosen on ventral side of the left upper forearm. One hour before the start of functional tests, 2 g of EMLA® cream were placed on one skin site, followed 20 min later by cream placement on another site, followed 20 min later by cream placement on a third site. On each treated area, the tip of a 25-gauge needle was introduced 3 mm intradermally, mimicking the initial needle insertion performed for microdialysis. The four sites were then instrumented for measurement of skin blood flow using laser Doppler flowmetry. After baseline was recorded, postocclusive hyperemia (5 min) and thermal hyperemia (35 min) were performed. At the end of the experiment, maximal skin blood flow was achieved by heating to 44°C during 10 min. Data are expressed as mean ± SD, and were analyzed with ANOVA for repeated measures, followed by Tukey test.
Results: We observed an initial dose dependent decrease in baseline, peak PORH, and peak thermal hyperemic cutaneous blood conductance, when EMLA® cream was applied for 40 and 60 min. Two hours after EMLA® removal, we observed a decreased baseline, post-occlusive hyperemia and a trend towards a decreased thermal peak for the 60 min sites. However, conductance values were similar to the control sites in the 20 and 40 min sites (see table)

Cutaneous vascular conductance (% max)	Control	EMLA® 20 min	EMLA® 40 min	EMLA® 60 min
Baseline	9 ± 6.8	7.5 ± 3.7	7.5 ± 5.3	5.3 ± 2[a]
PORH Peak conductance	39.5 ± 17	41 ± 26	38 ± 27	26 ± 10 [a]
Local heating initial peak	66.3 ± 14	67.2 ± 21	68.5 ± 10	58.7 ± 19
Local heating late plateau	83 ± 18	81.1 ± 33	87.1 ± 6	77.5 ± 10

A $P < 0.05$ vs. control (ANOVA $P < 0.05$).

Conclusion: EMLA® cream, when applied during 40 min, induces a significant 75% decrease in the pain following intradermal needle insertion, while not modifying skin postocclusive and thermal hyperemia 2 h after cream removal. Therefore, we recommend its use in such conditions before performing microdialysis coupled with laser Doppler flowmetry in cohort studies aimed at studying microvascular dysfunction in patients with microvascular diseases.

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The role of inflammation in smoking-related endothelial dysfunction: a cross-sectional study in humans

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Introduction: Smoking is a predominant cause of cardiovascular (CV) events in young adults. Direct toxicity of cigarette smoke components, as well as inflammatory and metabolic disturbances, can lead to endothelial dysfunction, reflected by lowered flow-mediated vasodilation (FMD), a marker of early atherosclerosis. The purpose of this study was to compare and analyse the determinants of brachial artery FMD in a subset of smokers and non smokers.
Methods: Current smokers ($n = 29$), non smokers ($n = 46$) and former smokers ($n = 22$) were consecutively included in the study, provided they were free from previous CV disease and any pharmacological treatment. They underwent CV risk assessement on the basis of traditional risk factors, 10-year Framingham risk calculation, metabolic (insulin, triglycerides) and inflammatory (high-sensitive CRP (hsCRP), leucocytes) parameters. FMD was measured as classically, with a semi-automated device ensuring high reproducibility.
Results: Current smokers had lower FMD (5.11%) than non smokers (6.35%) and former smokers (6.96%), $P < 0.05$. Other risk factors associated with low FMD were 10-year Framingham risk ($P < 0.01$), hsCRP ($P < 0.01$) and leucocytes ($P < 0.05$), at the exclusion of metabolic parameters.
In multivariate analysis (Table), FMD differences between groups were unchanged after adjustment for 10-year Framingham risk, whereas further adjustments for CRP or leucocytes attenuated or abolished the statistical significance of such differences.

	Adjusted *P* values for FMD differences between groups	
Adjustment for:	Current vs. non smokers	Current vs. former smokers
Framingham	<0.05	<0.01
Framingham and hsCRP	NS	<0.05
Framingham and leucocytes	NS	NS

Conclusion: This study supports the role of inflammatory mechanisms in the endothelial dysfunction of smokers, independently of traditional and metabolic risk factors. Such mechanisms may involve the local recruitment of leucocytes at the surface of endothelial cells.

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Protection of endothelial function by long term heart rate reduction induced by ivabradine in a rat model of chronic heart failure

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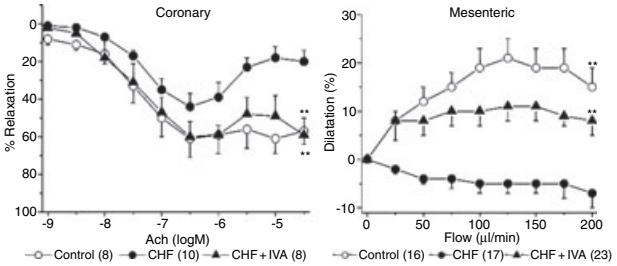
Introduction: We have shown previously that long-term selective heart rate reduction (HRR) with the I_f inhibitor ivabradine (IVA) improves cardiac function and remodeling in rat chronic heart failure (CHF), but whether HRR also affects endothelial function in CHF is unknown.
Methods: Thus, CHF rats (coronary ligation) were untreated or treated for 3 months with IVA (10 mg/kg/d in diet). Interventricular coronary arteries were mounted in a wire myograph (responses to acetylcholine, Ach), while small mesenteric resistance arteries were canulated to evaluate flow-mediated dilatation (FMD).
Results: Figure shows that IVA improved endothelial dysfunction in coronary and peripheral (mesenteric) arteries isolated from CHF rats (**: $P = 0.01$ vs. CHF). These effects were abolished by the NOS inhibitor LNNA (10^{-4} M). In nonprecontracted CHF coronary arteries (in the presence of LNNA 10^{-4} M), Ach induced concentration-dependent, endothelium-dependent contractions that were not affected by IVA. In CHF mesenteric arteries, the cyclooxygenase inhibitor diclofenac (10^{-5} M) improved FMD to a similar extend in untreated and IVA-treated rats.



Conclusion: Long-term HRR with IVA protects coronary and peripheral endothelial function in CHF, mostly through an improved endothelial NO production, without affecting the EDCF pathway.

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Cutaneous vascular responses to hypoxia, hypercapnia, and hyperpnea in humans

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Introduction: Little is known about the cutaneous sympathetic or vascular responses to hypoxia or hypercapnia in human non-acral skin. This study investigated the effects of acute hypoxia, hypercapnia, and the possible influence of hypoxic/hypercapnic hyperpnea on cutaneous vascular regulation.
Methods: Thirty-seven healthy subjects were instrumented with two microdialysis fibers in the ventral forearm. Each site was continuously infused with Ringer's (control) or bretylium tosylate (10 mM) to prevent sympathetically mediated vasoconstriction. Skin blood flow was assessed at each site (laser-Doppler flowmetry) and cutaneous vascular conductance (CVC) was calculated (red blood cell flux/mean arterial pressure) and scaled as percentage maximal CVC (local heating to 43°C). Adequacy of bretylium administration was verified via whole body cold stress.
Results: Thirteen subjects were exposed to 10 min 85% and 80% hypoxia, which caused a 25.8 ± 6.7% increase in CVC in the control site, and a 20.5 ± 3.9% increase in CVC in the bretylium site (both $P = 0.05$). There was no effect of drug on the magnitude of this response ($P = 0.40$). Ten subjects were exposed to hyperpnea (matching hypoxic increases in tidal volume, i.e 1.5 L) which caused no change in CVC in either site (both $P > 0.50$). Fourteen subjects were exposed to hypercapnia (end-tidal CO_2 5 and 9 torr above eucapnic levels), which caused a 14.4 ± 6.1% increase in CVC in the control site ($P = 0.16$), and a 6.4 ± 3.6% increase in CVC in the bretylium site ($P = 0.04$).
Conclusion: Thus, hypoxia causes cutaneous vasodilation that is not masked by sympathetic vasoconstriction, and this is not an effect of hyperpnea per se. Hypercapnia appears to have a mild vasodilatory effect on the cutaneous circulation.

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Effects of hyperlipidic diet (egg yolk) and hypothyroid drug (carbimazole) on atherosclerosis

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Introduction: *Psammomys obesus*, represents a powerful tool to follow the metabolic changes that occur under nutritional abundance and when energy

intake exceeds energy expenditure. The object of this study is to compare the effects of two atherogenic factors (hyperlipidic diet and hypothyroidism) and to evaluate some plasmatic, metabolic and structural disorders.
Methods: Three groups of psammomys were constituted: the 1st control group received daily for 6 months, natural diet (halophilous plant) (50 g/day). The 2nd received daily natural diet (50 g/day) and ¼ egg yolk (6 months). The 3rd group received daily standard laboratory diet (10 g/day) added with carbimazole (0.03%) dissolved in drinking water (5 months). Monthly, analysis of biochemical parameters is effectuated by the enzymatic method and electrophoretic analysis of lipoproteins by horizontal gel electrophoresis. For histological examination, the aortas were staining with trichromatic of Masson. For culture technique, aortic smooth muscle cells (aSMC) proliferation was analysed.
Results: Hypertriglyceridemia and hypercholesterolemia are registered in the two groups of experimental Psammomys but are more pronounced in Psammomys on natural diet with egg yolk, cholesterolemia showed in this last group one month after the beginning of the experimentation 2140 ± 88 mg/dl vs. 48 ± 2 mg/dl. The analysis of electrophoretic lipoproteins profiles revealed a reduction of 98% and 93% in HDL and an increase of 98% and 66% in atherogenic fractions, VLDL-LDL in Psammomys on hyperlipidic diet and Psammomys on hypothyroid state respectively. A major histological alterations of the thoracic aorta were exhibited in two groups of experimental Psammomys (blood aggregation, phenotypic modulation of aSMC, collagens accumulation). The strongest changes were particularly displayed on aortic sections in the 2nd group (cut of aortic segment). High level of smooth muscle cells proliferation in culture was recorded in two experimental groups.
Conclusion: The two groups of Psammomys showed plasmatic, structural and cellular atherogenic alterations. The development of nutrition induced diabetes and cardiovascular complications in this animal model mimics that of human populations emerging from a food scarce environment into nutritional affluence inappropriate for human metabolic capacity.

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Hyperlipidic diet with carbimazole induce dyslipidemia and atherosclerotic changes in the rat Wistar aorta

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Introduction: Hyperlipidic diet has often been used to elevate serum; or tissue cholesterol levels to study the development of atherosclerosis. Whereas Wistar rat is considered to be resistant to induction of hypercholesterolemia by hyperlipidic feeding. The aim of our study is to associate at this diet the Carbimazole in order to evaluate some plasmatic and histological disorders.
Methods: Sixteen Wistar rat (181.00 ± 36.63 g) were used in this study. Rats were divided in two groups. The first group, or control group (n = 8) was fed daily 15 g of the standard laboratory diet and water *ad libitum*. The second group (n = 8) received daily 15 g of standard laboratory diet added with a cooked egg yolk and 0.03% Carbimazole in their drinking water for 12 months. Plasma total cholesterol and triglyceride levels were measured using enzymatic Kits. Total hepatic lipid is determined by Folch method. After 12 months, rats were sacrificed; thyroid and aorta were fixed at Bouin for histological study.
Results: Hypothyroidism induced by Carbimazole is confirmed by histological of thyroid study, this show an increase of mass of thyroid gland. Histologically, the enlarged gland is characterized by proliferation of several components of thyroid gland, such as thyrocytes, fibroblasts, Parafollicular cells, enlarged blood capillaries and the components of extracellular matrixes.
At the end of experiment, plasma cholesterol and triglyceride levels such as total hepatic lipid were found to increase in experimental group as compared to the control group.
According to the light microscopic findings, the thoracic aorta showed characteristic views of atherosclerotic disease, in particular a sub intimal thickening as well as an important aggregation of blooding elements.
Conclusion: In conclusion hyperlipidic diet plus Carbimazole increase total cholesterol and triglyceride levels as well as significant alterations in the rat aorta.

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Interaction of myosin light chain kinase 210 with NF-κB pathway in endothelium is critical for lipopolysaccharide-induced vascular hyporeactivity

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Introduction: The present study was aimed to better understand the molecular mechanism of the protection against lipopolysaccharide (LPS) -induced vascular alterations by deletion of the long isoform of myosin light kinase (MLCK210), using MLCK210$^{-/-}$ mice. The hypothesis of a probable functional association of MLCK210 with proteins belonging to the NF-κB family that might be responsible for vascular hyporeactivity was also tested.
Methods: Vascular reactivity was assessed in aortic rings from MLCK210 wild type and knock out mice pre-incubated with or without lipopolysaccharides (LPS) (100 mg/ml) for 20 h (n = 8). Nitric oxide (NO) spin trapping with electronic paramagnetic resonance techniques (n = 5) and immuno-histochemical studies (n = 3) were conducted. Western blotting (n = 4) and immuno-precipitation of MLCK210, NF-κB and p-IκBα (n = 3) were performed in human endothelial cells, Eahy 926. Statistical analysis were performed by two-way ANOVA for myography and by unpaired Student's t-test for NO spin trapping and western blot analysis.
Results: We provide evidence that MLCK210 deletion prevents LPS-induced vascular hyporeactivity but not endothelial dysfunction in the aorta *in vitro*. Deletion of MLCK210 inhibits NF-κB activation by 29% and the increase of NO release by 71% *via* induction of inducible NO synthase within the vascular wall. Of particular interest is the demonstration that in human endothelial cells, EAHY.926, LPS-induced NF-κB activation occurs via increase of 23% of MLCK activity sensitive to the MLCK inhibitor, ML-7, and physical interactions between MLCK210 with both NF-κB and p-IκBα. We report the first time that NF-κB and p-IκBα are novel partners of MLCK210 within endothelial cells.
Conclusion: The present study and our previous work showing that MLCK210 is involved in lethal complications as well as in vascular reactivity changes associated with endotoxin shock *in vivo* demonstrate a pivotal role of MLCK210 in vascular inflammatory pathologies.

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Increased procoagulant and platelet microparticles account for endothelial dysfunction in patients with metabolic syndrome

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Introduction: Microparticles are membrane vesicles with procoagulant and proinflammatory properties released during cell activation. Elevated levels of microparticles have been reported in many cardiovascular diseases. Here, we have characterized the cellular origins and studied the effects on endothelial cells of circulating microparticles from healthy subjects and patients with metabolic syndrome (MS), which is referring to the clustering of several cardiometabolic risk factors, including abdominal obesity, hyperglycaemia, dyslipidaemia and elevated blood pressure.
Methods: Microparticles were obtained from whole blood of healthy (n = 22) and MS subjects (n = 27) by serial centrifugations. Then, their counts and cellular origins were determined by flow cytometry in platelet-free plasma. Human endothelial cell line Eahy 926 was treated for 24 h with circulating concentration of microparticles and subjected to measurement of both nitric oxide (NO) and superoxide anion (O_2^-) by electronic paramagnetic resonance technique. Enzymes linked to NO pathway were also analyzed by Western Blotting. Statistical analysis were performed by a one way analysis of variance (ANOVA), and Mann–Whitney U tests. $P < 0.05$ was considered to be statistically significant (n = 5–6).
Results: Patients with MS displayed increased circulating levels of microparticles compared to healthy subjects (14718.4 ± 4095.5 vs. 5770.2 ± 2029.9 events/μL of plasma). Levels of platelets-derived ($CD41^+$) and procoagulant microparticles (Annexin V^+) were also increased in MS patients by 1.9 and 4.7-fold, respectively. However, microparticles derived from endothelial cells ($CD146^+$), granulocytes ($CD66b^+$), erythrocytes ($CD235^+$) and leucocytes ($CD45^+$) were not significantly different between MS and healthy subjects. *In vitro* treatment of Eahy 926 cells with microparticles from MS patients but not from healthy subjects reduced both NO and O_2^- productions by 51% and 42%, respectively. Microparticles from healthy subjects did not affect endothelial NO-synthase (eNOS) expression, enhanced its phosphorylation at both activator and inhibitor sites in identical manner and did not modify caveolin-1 expression. Microparticles from MS also did not change eNOS expression or its phosphorylation at the activator site S1177 but they markedly enhanced its phosphorylation at the inhibitor site Thr495. Finally, MS microparticles did not modify caveolin expression.
Conclusion: In conclusion, we demonstrate that patients with MS displayed increased circulating microparticles especially those originated from platelet and procoagulant microparticles. Interestingly, microparticles from MS reduce NO bioavailability rather by a mechanism independent to an increase of oxidative stress in endothelial cells via reduction of eNOS activity that probably accounts for endothelial dysfunction. Taken together these results highlight a relationship between increased circulating of procoagulant and platelet microparticles with endothelial dysfunction in MS.

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New monitoring software for larger clinical application of brachial artery flow-mediated vasodilation measurement

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Introduction: Flow-mediated vasodilation (FMD) of the brachial artery (BA), a non invasive marker of endothelial function in human, is widely accepted for evaluating cardiovascular risk-reduction therapies. However, its clinical use is limited by operator dependence of most measurement methods.
Methods: A new home-made automated computerized analysis of BA ultrasound providing a continuous evolution of the diameter during acute hyperemia was tested in 10 normal volunteers and 26 asymptomatic subjects with at least one cardiovascular risk factor. FMD was the percentage of the maximum hyperemic diastolic diameter from baseline. Within reading variability in diameters and FMD were assessed by reading one scan from the same subject by two observers. Within subject variability was assessed by analysing two repeated measurements of the same subject 1 hour apart (short-term), and 1 week or 1 month appart (long-term).
Results: Thanks to very low coefficients of variation of diameter measurements (<2% for within reading, 4–8% for within subjects variabilities), FMD variability was as low as 7% on average for within reading and 8–18% for within subject variabilities. Short- and long-term FMD variabilities were twice higher in at risk subjects than in healthy volunteers.

Variability:	*Coefficients of variation of FMD measurements*	
	Healthy volunteers (n = 10)	At risk subjects (n = 26)
Within reading	7.5	6.9
Short-term within subject	7.8	16.5
Long-term within subject	9.6	18.1

Conclusion: This method overcomes the 35–50% variability of FMD measurement seen with conventional manual analysis in normal volunteers and in patients with major cardiovascular risk factors, thus supporting its applicability in clinical trials for patients with disease conditions.
A simple calculation indicates that, for a parallel group or a cross-over design, our new method requires two to three times less subjects to include than conventional methods.

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Influence of pravastatin on carotid artery structure and function in HIV-infected patients under antiretroviral therapy

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Introduction: Dyslipidemia with accelerated atherosclerosis is an emerging complication in HIV-infected (HIV+) patients treated with antiretroviral therapy (HAART). The effect of statins on atherosclerosis in this population remains unknown.

Methods: We investigated the impact of pravastatin therapy (P) on carotid intima-media thickness (IMT-CCA) and stiffness, aortic stiffness and pulse pressure (PP) in HIV+ patients with hypercholesterolemia (LDL-cholesterol ^{3}160 mg/dL) treated with HAART ($\geqslant$12 months). With a predefined calculation of the sample size, 42 patients treated with P ($\geqslant$12 months) and 42 age, sex and smoking status-matched hypercholesterolemic patients without lipid-lowering treatment were enrolled. IMT-CCA and PPwere determined usinganon invasivehigh-definition echotracking device and applanation tonometry in a central core laboratory blinded to treatment.

Results: Both groups were similar for clinical characteristics including cardiovascular risk factors and HIV parameters. Mean duration of HIV infection was similar among P and P never treated patients 12 ± 4 vs. 11 ± 5 years, $P = 0.24$). The mean duration of dyslipidemia was higher in the P group (3.0 ± 2 vs. 1.7 ± 1.4 years, $P = 0.004$). Patients were treated by P (mean dosage: 30 ± 10 mg /day) with a mean duration of 23 ± 8 months. Pravastatin did not influence carotid artery structure nor function; No difference was observed in the IMT CCA between HIV-infected patients under pravastatin and controls (689 ± 131 vs. 717 ± 148 mm, $P = 0.36$). Aortic stiffness measured using the carotid-femoral PWV did not differ between the two groups (9.6 ± 1.7 vs. 10.0 ± 1.8 m/s, $P = 0.25$).

Using logistic regression, determinants of IMT were age and carotid PP whereas age, duration of HIV infection, abdominal perimeter and lypodystrophic syndrome determined aortic stiffness.

Conclusion: The use of pravastatin was not associated with a significantly lower carotid IMT in dyslipidemic HIV+ patients. Randomised trials with statins are needed to confirm if HIV-related atherosclerosis is resistant to pravastatin or to all statins.

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Circulating microparticles from septic shock patients modulate tissular expression of enzymes related to inflammation and oxidative stress

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Introduction: Septic shock is associated with hypotension and multiple organic failure system after infection by microorganisms. High levels of circulating microparticles (MPs) have been found in plasma from septic patients. MPs are small vesicles released from plasma membrane of activated or apoptotic cells. Here, we have investigated the effects of the injection of MPs from septic or healthy subjects in mice on tissular protein expression.

Methods: MPs were extracted from whole blood of septic ($n = 32$) and normal ($n = 16$) subjects that were utilized as controls, following serial centrifugations (for cell origin of MPs, please see the abstract Mostefai et al.). Both types of MPs have been injected i.v. in mice, and after 24 h, mice were sacrificed and heart, lung, kidneys and liver were dissected and homogenized, for western blot assays. Immunoblots were quantified by densitometry and results were normalized compared to respective controls. Also organs were used for NO and superoxide anion (O_2^-) measurements by electronic paramagnetic resonance.

Results: O_2^- production was greater in heart and liver (2.33 and 2-fold respectively) but not in other tissues from mice treated with septic MPs vs. healthy subjects MPs. Tissular NO contents were not significantly different in the two groups except that of liver in which septic MPs treatment induced 2-fold increase of NO compared to control MPs.

	eNOS	iNOS	COX-1	COX-2	p-IκBα	p22^{phox}	Mn SOD	Cu/Zn SOD	EC SOD
Heart	169 ± 58*	107 ± 23**	69 ± 5***	ND	86 ± 40	89 ± 60	64 ± 40	100 ± 60	204 ± 22***
Lung	76 ± 26*	42 ± 23	55 ± 27	14 ± 16*	33 ± 32	ND	23 ± 24	26 ± 28	-20 ± 27
Kidney	-50 ± 10*	11 ± 23	-2 ± 1	-1 ± 6	-33 ± 10*	ND	4.5 ± 9	18 ± 12	29 ± 18
Liver	-25 ± 8*	4 ± 14	5.3 ± 5	85 ± 9	-37 ± 6***	ND	-9 ± 2*	6.5 ± 1**	-33 ± 13

*$P = 0.05$, **$P = 0.01$,***$P = 0.001$ using unpaired Student's t test. ND: not determined.

Conclusion: In conclusion, septic MPs differentially affect the expression of proteins leading both to nitrosative and oxidative stresses, the main changes occurring in heart and liver.

Table: Western blot analysis of proteins from tissues from microparticles-treated mice. Results are expressed as% of increase or decrease of level expression for each protein from tissues from septic microparticles- vs. healthy subject microparticles-treated mice.

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Circulating endothelial cells as a marker of vascular damage in patients with cardiovascular risk

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Introduction: Measurement of circulating endothelial cells (CECs) has been considered as a simple method to evaluate a vascular injury. Several clinical studies have demonstrated increased endothelaemia in patients at high cardiovascular (CV) risk and also in some non-cardiovascular disorders. The aim of this study was to determine the number of CECs in patients with acute coronary syndrome (ACS) and advanced peripheral arterial occlusive disease (PAOD) of lower limbs and to compare with a control group.

Methods: The study involved 36 hospitalized patients [10 patients with ACS treated by standard therapy; 10 patients with advanced PAOD treated by PGE_2. Seven patients with surgical intervention, nine patients without CV diseases as controls]. Quantitative measurement of endothelial cells (ECs) in blood was performed by the method according to Hladovec (1) based on counting of ECs in Buerker's chamber. The isolated ECs were calculated after removal of platelets by addition of adenosine-diphosphate (2).

Results: It was found that the number of ECs was significantly higher in patients with increased cardiovascular risk, ACS (4.9 ± 1.59) and PAOD (3.74 ± 0.61) when compared with controls (1.38 ± 0.899; $P < 0.05$). ECs number in patients with PAOD significantly increased after surgery (3.88 ± 0.77) in comparison before intervention (2.67 ± 0.86; $P < 0.05$).

Conclusion: This pilot study involving relatively small number of patients demonstrates significant increase of endothelaemia in patients at high CV risk, which is consistent with other available data. Usage of immunomagnetic methods for ECs measurement will enable an improvement of screening and diagnostics in clinical conditions.

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Fourier transform spectral analysis of cutaneous blood flux in systemic sclerosis

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Introduction: Endothelial dysfunction is an early event and a critical step in the pathogenesis of systemic sclerosis. Accurate and sensitive tests testing this microvascular endothelial dysfunction are required. Spectral analysis of skin blood flow contains a characteristic low frequency reported to be associated with endothelial function in healthy subjects. We tested the hypothesis that the relative amplitude of the oscillation for this frequency spectrum from 0.008 to 0.021 Hz would be less important in patients with systemic sclerosis than healthy subjects and patients with primary Raynaud's phenomenon.

Methods: Twenty-one patients with systemic sclerosis, 20 patients with primary Raynaud phenomenon and 11 healthy subjects were enrolled. Skin perfusion was recorded at rest on the third left finger pad during 30 minutes by laser Doppler flowmetry. With Fourier transform spectral analysis, the mean amplitude of cutaneous blood perfusion signal of the total spectrum from 0.008 to 1.6 Hz and the mean amplitude of each characteristic frequency in the laser Doppler flowmeter blood flow oscillations were calculated.

Results: Relative amplitude of each characteristic frequency in the laser Doppler flowmeter blood flow oscillations were not statistically different in the three groups, particularly for frequency spectrum from 0.008 to 0.02 Hz.

Conclusion: Fourier transform spectral analysis of baseline cutaneous blood flow does not provide significant information. Further studies are required using wavelet spectral analysis, or under stimulated conditions.

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Circulating microparticles from septic chock patients exert protective role on vascular function

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Microparticles (MPs) are membrane vesicle harbouring cell surface proteins and containing cytoplasmic components of the original cell. Elevated levels of circulating MPs have been detected in pathological states associated with vascular dysfunction. The present study was designed to characterise MPs from both septic shock and healthy subjects and to investigate their effects on vascular functions.

MPs were obtained from whole blood of patients ($n = 32$) or healthy ($n = 16$) subjects by serial centrifugations. Then, their counts and cellular origins are determined by flow cytometry in platelet-free plasma. Markers of endothelial (CD146), platelet (CD41 and P-selectin: CD62P), and leukocyte (L-selectin: CD62L) activation were measured. Nitric oxide (NO) and superoxide anion (O_2^-) were measured by electronic paramagnetic resonance technique (ERP). MPs either from patients or healthy subject were injected *i.v* to mice and then, vascular reactivity was assessed in aorta by myography. Statistical analyses were performed by Mann–Whitney U-test for MPs measurement, two-way analysis of variance (ANOVA) for myography and Student's *t*-test for ERP.

Flow cytometry analysis from patients with septic shock indicated that circulating levels of MPs were increased compared with healthy subjects (14080 ± 2649 vs. 9587 ± 3736) ($P < 0.05$). Endothelial- (192.3 ± 35.6 vs. 73.5 ± 9.4) ($P < 0.01$) and platelet-derived MPs (13290 ± 2643 vs. 8840 ± 3753) ($P < 0.05$), as well as P-selectin$^+$ (1404 ± 32.6 vs. 48.6 ± 12.4) and L-selectin$^+$ MPs (61.4 ± 16.4 vs. 15.75 ± 5.5) ($P < 0.05$, $P < 0.01$, respectively) were increased in septic patients. No difference in NO or O_2^- production between mouse aortas treated with MPs from either septic of healthy subjects. Interestingly, the sensitivity of contraction in response to serotonin was increased in aorta taken from mouse treated with septic MPs (EC_{50}: 75 ± 2.1x10^{-8} M) ($n = 7$) compared to those with healthy subjects (EC_{50}: 480 ± 090 nM) ($n = 6$) ($P = 0.01$). This effect was not modified in presence either of NO-synthase inhibitor, nitro-L-arginine or cyclooxygenase (COX)-2 inhibitor, NS398. In other hand, the non-selective COX inhibitor, indomethacin, either reduced (50% of inhibition) or abolished (94% of inhibition) contraction to serotonin in aorta from mice treated with healthy ($n = 4$) and septic MPs, ($n = 5$) respectively, $P < 0.05$. Contraction to $CaCl_2$ on arteries exposed to KCl-depolarization ($n = 3$) and relaxation to the Rho-kinase inhibitor ($n = 4$) on vessels pre-contracted with U46619 were identical in aorta taken either from healthy- or septic-treated mice.

In conclusion, we demonstrate that septic patients displayed increased circulating of MPs especially those originated from non activated and activated platelet, endothelial and activated leucocytes. Interestingly, septic MPs *in vivo* enhanced reactivity to serotonin without affecting nitrosative or oxidative stresses within the aorta. This potentiating effect was linked to enhanced participation of vasoconstrictor metabolites from COX-1. The mechanisms implicated were not linked to enhance calcium entry or sensitization through the Rho-kinase pathway. Thus, septic MPs may be rather protective against vascular hyporeactivity accounting for hypotension in septic shock patients.

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Inhibition of ischemia-induced cell death with the protein TAT-BIR3/RING provides a new window of cardioprotection during reperfusion

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Introduction: Current investigated cardioprotective strategies against myocardial infarction are performed within the first minutes of reperfusion. We hypothesized that this short window at reperfusion could be timely increased using the X-linked inhibitor of apoptosis protein (X-IAP), the most effective endogenous inhibitor of apoptosis. X-IAP possesses four domains, i.e., BIR1, BIR2, BIR3 and RING. Among them, BIR3 and RING domains are known to interact with caspase-9 and caspase-3, respectively. Accordingly we investigated whether BIR3/RING administered using the TAT-fusion system, i.e. the biotherapeutic protein PTD-BIR3/RING, could be used as a cardioprotective agent against myocardial infarction at reperfusion.

Methods: We fused the C-terminal part of X-IAP (BIR3/RING) to the protein transduction domain (PTD) of HIV1 transactivator of transcription domain which confers to the protein (PTD-BIR3/RING) the ability to cross any cell membrane. After anesthesia, 6–8 weeks-old C57/BL6-J mice were subjected to 30 min coronary artery occlusion followed by 24 h coronary artery reperfusion (CAR). Intravenous injections of saline (control) or 0.8 μg/g of PTD-BIR3/RING were performed at 30 min, 3 h or 6 h after the onset of CAR. Caspases activites and the density of several pro- and anti-apoptotic proteins were assessed.

Results: In control mice, IS (TTC staining) reached 44 ± 4% of the area at risk after 24 h CAR. Administered at 30 min and 3 h during CAR, PTD-BIR3/RING significantly reduced IS (27 ± 4% and 29 ± 4%, respectively). This cardioprotection was lost when the protein was administered at 6 h CAR. We verified with immunohistochemistry that the protein was present within the cardiomyocytes. Concomitantly, caspases 3, 8 and 9 activities were inhibited and TUNEL-positive cardiomyocytes were reduced by 50% vs. control. Furthermore, Akt and Bad phosphorylation increased while Bax and truncated Bid expression decreased in PTD-BIR3/RING treated groups.

Conclusion: These results demonstrate that potent inhibition of apoptosis with PTD-BIR3/RING, an original product of biotherapy, reduces myocardial infarct size even when administered late during reperfusion. These results also suggest that beyond the first minutes of reperfusion, a new window of cardioprotection exists within the first hours of reperfusion.

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Evaluation of cardiac function with isolated working mice heart model after *in vivo* anthracycline treatment

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Introduction: Anthracyclines, such as doxorubicin (DXR) and epirubicin (EPI), are very effective anticancer drugs used to produce regression in a variety of haemopoietic and solid tumors in children and adults. However, the repeated clinical use of these drugs is limited by a high incidence of cardiotoxicity. Using the model of isolated working mice hearts, we aimed to assess the effect of *in vivo* anthracyclines administration on the cardiac function evaluated by an *in vitro* approach.

Methods: Female Swiss mice were treated with a single i.p. dose of 20 mg/kg EPI or DXR. Control mice received identical volume of 0.9% NaCl. Cardiac troponin I (cTnI) was measured in the plasma and the hearts were isolated and perfused in the working method 24 hours or 7 days after the treatment. Myocardial functional parameters (left ventricular developed pressure, contractility and relaxation index, aortic flow, coronary flow and heart rate) were recorded throughout the protocol. Cardiac response of isolated working hearts to an inotropic β-agonist was evaluated by the administration of 1 μM isoproterenol (ISO).

Results: General physical condition of mice was altered 7 days after the treatment of EPI or DXR (decrease of 13% of body weight and of heart/body weight ratio) while these modifications were not observed 24 hours after the treatment. Plasma cTnI levels were significantly increased in EPI or DXR-treated mice as compared with control mice ($P < 0.001$). However, the basal cardiac functional parameters and the contractile responsiveness to ISO infusion were similar in the different groups: control, EPI and DXR 24 hours and 7 days after the treatment.

Conclusion: Our results indicate that the usual anthracyclines' cardiotoxicity model described in the literature (single dose, i.p., 20 mg/kg) is associated with a significant general toxicity in mice 7 days after the treatment. However, the cardiac performances evaluated *in vitro* by the isolated working heart method were preserved in anthracyclines-treated mice while an increase in a marker of myocardial damage (cTnI) was detected. These unusual observations could be explained by modifications of cardiovascular functions occurring *in vivo* associated with a possible renal failure induced by anthracyclines.

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Role of selenium in cardiac lesions caused by neuroleptics

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Introduction: Neuroleptics can induce organic or functional heart lesions that may lead to the patient's death. The present study was undertaken to determine whether neuroleptics-induced lesions are correlated with blood and tissue selenium levels.

Methods: Twelve NZW rabbits were treated with levomepromazine (3 mg/kg/d) and risperidone (1 mg/kg once every other week) for 3 months and compared to 12 controls. At the end of the treatment period, selenium was measured in the blood and the heart, liver and kidneys of all animals. In addition, the hearts were examined histologically.

Results: There was a statistically significant ($P < 0.001$) 20% decrease in blood selenium levels in treated animals compared to controls. Myocardial selenium levels were twice lower in treated animals ($P < 0.001$) compared to controls, whereas liver and kidney levels were not different across experimental groups. In contrast to control animals, treated animals developed heart lesions including disorganization of cardiac fibers, myolysis, interstitial and endocardial fibrosis, and necrosis.

Conclusion: Our results support the hypothesis of a correlation between neuroleptics-induced heart lesions and decreased blood and myocardial selenium levels.

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Impact of somatostatin analogs on heart a meta-analysis

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Introduction: Cardiomyopathy may complicate acromegaly. Treatment with somatostatin analogs have been shown to improve some cardiac parameters but most published clinical trials involved few patients, are not randomized controlled studies and their results are variable. The aim of this study was to conduct a meta-analysis to obtain a more reliable picture of the effect of somatostatin analogs on heart in patients with acromegaly.

Methods: We systematically reviewed all studies of somatostatin analog in acromegaly. Eighteen studies were identified in three databases. We conducted a combined analysis of somatostatin analog effects using the overall effect size to evaluate significance and computing the weighted mean differences with and without treatment to assess effect size.

Results: Somatostatin analog treatment was associated with a significant decrease in heart rate [-5.8 (2.1) beats/min], left ventricular mass index [-22.3 (6.7) g/m^2), inter-ventricular septum thickness [-0.3 (0.2) mm), left ventricular posterior wall thickness [-0.8 (0.4) mm], ratio of E-wave and A-wave peak velocities of the mitral flow profile [0.2 (0.1)] and improvement in exercise duration [1.6 (0.4) min]. A beneficial trend was noted for left ventricular end-diastolic dimension [-1.5 (2.2) mm] and left ventricular ejection fraction [3.3 (1.7) %]. Overall effect sizes were not significant for blood pressure, left ventricular end-systolic dimension and fractional shortening. Better improvements were observed in studies with large IGF-I decreases or with large GH decrease and in studies with younger patients.

Conclusion: This meta-analysis confirms that somatostatin analog treatment aiming to achieve stringent control of serum GH/IGF-I concentrations is associated with significant positive effects on morphological and functional hemodynamic parameters in patients with acromegaly.

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Involvement of P2Y11 and P2Y6 receptors in pyridoxal 5′-phosphate (PLP)-induced cardiac preconditioning

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Introduction: In freshly isolated adult cardiomyocytes, 1–50 μM PLP prevents the ATP-induced increase in $[Ca^{2+}]_i$ in a concentration-dependent manner (1). Neonatal rat heart cells in culture treated with 10 μM PLP at the onset of a prolonged simulated ischemia are protected against creatine kinase (CK) release with a concomitant increase in intracellular PLP concentrations [PLP] i (2). Low plasma PLP confers an independent risk for coronary artery disease (3). The present study was designed to test whether physiological PLP concentrations may prevent myocardial infarction by pre-emptive preconditioning of the heart.

Methods: After 6-hydroxydopamine pretreatment and a 15-min stabilization period, isolated rat hearts were perfused for 25 min then subjected to 40 min of global ischemia and 30 min of reperfusion (I/R); exposed for 15 min to 0.05 μM PLP bracketed for 25 min with antagonists of P2 receptors (suramin, PPADS, AMPαS or MRS2578), 1 μM each, followed by a 5-min PLP-free perfusion before I/R; treated during 25 min with either glibenclamide (1 μM), 5-hydroxydecanoic acid (5-HD, 100 μM), U73122 (0.5 μM), H89 (1 μM) or KN93 (1 μM), with an infusion starting 5 min before PLP. The main endpoints of cardioprotection were the rate-pressure product (RPP), CK release and myocardial infarct size.

Results: Recovery of RPP, measured 15 min after reperfusion, was improved by PLP, blocked by the P2 antagonists, and decreased with the different inhibitors. Fifteen minutes after the end of ischemia, CK release reached maximal values in all groups. PLP provided significant protection whereas the P2 antagonists, 5-HD, a mitochondrial selective K_{ATP} antagonist, and glibenclamide, a nonselective K_{ATP} channel blocker suppress the protective effect on myocardial injury. The suppression of the cardioprotective effects of PLP by AMPαS, the PKA inhibitor (H89) and phospholipase C (PLC) blocker (U73122) is in agreement with the P2Y11 receptor being a receptor for PLP-induced preconditioning. The suppression of the cardioprotective effects of PLP by MRS2578 and U73122 is in agreement with the P2Y6 receptor being a receptor for PLP-induced preconditioning. Myocardial infarct size measurements were well correlated with CK release.

Conclusion: 1) PLC, PKA and CaMKII are implicated in the trigger phase of PLP preconditioning 2) activation of P2Y11 and P2Y6 receptor is required for PLP-induced cardioprotection.

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Sudden cardiac death in professional sports-persons

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Introduction: To compare anabolic steroid-induced cardiac lesions in professional sports-persons and in a rabbit model.

Methods: Out of 15 000 forensic autopsies performed on coroner's orders over a 24-year period (Jan 1981–Dec 2003) in the area of Lyon, France (population: 2 000 000), WHO criteria identified 2 250 cases of unexpected sudden cardiac death. Among these, 120 were found to have occurred during recreational sport and 12 in professional sports persons. In the latter category, the associated cardiac lesions were primitive: natural in six cases, and, according to inquest findings, induced by anabolic steroids in the other six. To shed light on the induced lesions, animal experiments were performed, administering Norethandrolone to rabbits which were then sacrificed and subjected to pathologic examination and caspase-3 assay by fluorometry on cardiac fragments.

Results: The natural primitive lesions were classical for such cases. The anabolic steroid-induced lesions comprised coronary thrombosis associated with left ventricle hypertrophy and lesions analogous to toxic or adrenergic myocarditis. The same lesions were found, to varying degrees, in the rabbit models, which showed significantly elevated Caspase-3 activity as compared to controls.

Conclusion: Anabolic steroids would seem, to varying degrees, to induce lesions analogous to those found in myocardiopathy and toxic myocarditis. Their elevated Caspase-3 activity makes these lesions apoptotic in nature. Controlling or banning doping in professional sports being unfeasible in the present state of affairs, associating apoptosis inhibitors might hold out hope of limiting the incidence of severe evaluative cardiac lesions.

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Effect of age on treatment practices in patients with heart failure and preserved ejection fraction

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Introduction: Despite heart failure (HF) with preserved left ventricular ejection fraction (EF) being common in the elderly, very few intervention trials have been initiated in this specific population. One of the objectives of the ETICS study was to evaluate medical treatment at discharge and after 1 year in patients hospitalised for a first episode of HF in 2000. We report the results concerning treatments of elderly (≥75 years) and non-elderly patients (<75 years) with preserved EF at discharge and at 1 year.

Methods: We studied a cohort of 263 patients hospitalised for a first episode of HF with preserved EF. Of these patients, 150 were ≥ 75 years (66 ± 8 years, 44% women) and 113 were < 75 years (81 ± 5 years, 63% women). Overall mean EF was 63 ± 8%. The main aetiologies in the two groups were hypertension (63%, 58%) followed by ischaemic heart disease (32, 26%). Medical treatment records were complete at discharge and at one year after discharge.

Results: At discharge, as at 1 year after discharge, diuretics were the drugs most commonly prescribed in the two groups of elderly and non-elderly patients (82% and 80% at discharge, and 81% and 75% at 1 year, respectively), followed by ACE inhibitors (46% and 52% at discharge, and 47% and 44% at 1 year), beta-blockers (27% and 28% at discharge, and 27% and 30% at 1 year), calcium channel blockers (28% and 27% at discharge, and 26% and 27% at 1 year), spironolactone (15% and 29% at discharge, and 23% and 29% at 1 year), cardiac glycosides (20% and 19% at discharge, and 24% and 25% at 1 year), and angiotensin II receptor antagonists (4% and 6% at discharge, and 5% and 6% at 1 year). Age did not influence drug prescription rates at discharge or at 1 year, except for the spironolactone prescription rate, which decreased at discharge in elderly patients. At discharge, ACE inhibitor and beta-blocker daily doses were lower in older patients, while, at 1 year, no differences in daily doses of these drugs were observed between patients above and below the age of 75 years.

Conclusion: Loop diuretics are largely prescribed, followed by ACE inhibitors in elderly and non-elderly patients with HFand preserved EF. Once prescribed at hospital discharge, main prescription rates did not change significantly over time except for the spironolactone prescription rate, which decreased at discharge in older patients. Age did not influence daily dosages at discharge or at one year, except for the ACE inhibitor and beta-blocker daily doses, which were lower at discharge in elderly patients. The present study emphasizes the need for therapeutic studies in elderly patients with HF with HF and preserved EF.

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Combination of B-type Natriuretic Peptide and MIBG wash out rate improves risk stratification in patients with advanced heart failure

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Introduction: Autonomic nervous system dysfunction is common in congestive heart failure (CHF) and is believed to predispose patients to an increased risk of death. Our primary objective was to assess differences in MIBG scintigraphy results between survivors and non survivorsin patient with advanced heart failure. Our secondary objective was to identify the prognostic value of MIBG scintigraphy in advanced heart failure alone or in combination with other prognostic factors.

Methods: Patients with advanced heart failure (class NYHA 3 or 4) underwent prospectively MIBG scintigraphy and clinical, biological as morphological assessment of their heart failure status The heart /mediastinum MIBG uptake ratio (at 2 and 4 hours) and the wash out rate (WOR) were calculated. All patients were followed up until 1/11/2006 and their vital status was assessed through phone calls or outpatient department visit. We performed an univariate analysis between survivors and non survivors. We performed a cox proportional hazard regression to assess the MIBG scintigraphy related-cut off able to identify patient at risk of death. Finally we combined scintigraphy markers with other parameters to enhance the prognostic value of MIBG scintigraphy.

Results: The study population included 38 patients (mean age: 47 ± 10 years), with mean NYHA class 2.8, ejection fraction of 23 ± 11%. End-diastolic diameter was enlarged 68 ± 11 mm and neurohormones level high (BNP: 847 pg/ml and norepinephrine 632 pg/ml). During a follow up of 14 ± 10 months, six patients (16%) died. In univariate analysis only BNP was different and higher in non survivors (1738 vs. 700 pg/ml, $P = 0.01$) and the only predictor for mortality ($\chi^2 = 7.49$, $P = 0.006$) using a cox proportional hazard regression. Heart / mediastinum ratio MIBG uptake at 2 and 4 hours tend to be lower in non survivors. The 2 year survival rate in patients with a WOR > 43% (median value) was 77% when plasma BNP was > 603 pg/mL (median value), compared with 86%, when plasma BNP was ≤ 603 pg/mL ($P = 0.04$).

Conclusion: Plasma BNP provides additive independent prognostic information compared to WOR alone in patients with advanced heart failure.

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Central action of native angiotensin II on spontaneous baroreflex responses in the trout Oncorhynchus mykiss

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Introduction: Cardiovascular baroreceptor control evolved early during evolution and in teleost fish, baroreflex mediated modulation of heart rate (HR) is exclusively cholinergic. Consequently, teleost fish are interesting models to investigate the central action of regulatory neuropeptides upon the cardiac parasympathetic limb of the autonomic nervous system. In the present study, we investigate the central action of trout angiotensin II (ANG II) on the spontaneous baroreflex sensitivity (BRS) in our animal model, the unanesthetized trout.

Methods: The animals were equipped with two subcutaneous electrocardiographic (ECG) electrodes, a dorsal aorta catheter and an intracerebroventricular (ICV) cannula which was inserted within the third ventricle of the brain. The ECG and the systolic blood pressure (SBP) signals were recorded during 30-min recording sessions and all injections were made at the fifth minute of the test. The time-series were processed with a sequence technique in order to detect the sequences of three or more consecutive increases in the SBP pulse, or three or more decreases in the SBP pulse correlated respectively with one delay beat increase of the RR interval of the ECG signal or shortening of this interval. The slope of the average regression line between the SBP and the RR intervals for each type of sequence was taken as a measure of the spontaneous BRS.

Results: ICV injection of vehicle (0.5 μL) had no effect on HR, SBP, the total number of positive or negative sequences or on the spontaneous BRS. By contrast, ANG II at doses of 5 and 50 pmol increased HR but only 50 pmol ANG II elevated SBP. For all doses, ANG II reduced the spontaneous BRS, but the peptide had no effect upon the number of each baroreflex sequences. Intra-arterial injections of atropine abolished all baroreflex sequences confirming that the autonomic control of the cardiac BRS was solely due to vagal parasympathetic control. After atropinization, the ICV injection of 5 pmol ANG II had no effect upon HR, SBP and the baroreflex parameters.

Conclusion: This study determines for the first time in a non-mammalian species spontaneous baroreflex responses and demonstrates that within the brain of teleost fish, ANG II reduces the sensitivity of the baroreflex responses through a probable control of the vagal parasympathetic activity.

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First-generation sedating antihistamines: prone to cause torsades de pointes?

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Introduction: Potential cardiac toxicity of second-generation nonsedating antihistamines has largely been carefully examined during their development. It has been demonstrated that certain nonsedating antihistamines such as terfenadine and astemizole have potential to prolong the QT interval, which predispose to the development of Torsades de Pointes (TdP) and sudden deaths.

First-generation sedating antihistamines such as hydroxyzine (diphenylmethane derivates) and alimemazine (phenothiazines with antihistaminic properties and no antipsychotic properties) are widely prescribed for the treatment of anxiety and insomnia in psychiatric patients. Although it seems that ability to cause QT prolongation is not a class effect, this potential cardiac toxicity questions us on the capacity for the first-generation sedating antihistamines to expose to QT prolongation.

Methods: The current study was designed to determine if hydroxyzine and alimemazine are responsible for QT interval lengthening. We studied 350 patients admitted in a psychiatric unit. For each of the patients, a 12-lead electrocardiogram recording (ECG) was performed and QT measurements were corrected by Bazett's formula (QTc). Patients were categorized into different groups based on the presence or not of hydroxyzine or alimemazine in the patients' treatments. The comparaison of QTc values distributions of the different groups by using Mann–Withney U-test.

Results: Participants with missing treatments information or ECG were excluded from the analysis. Eleven patients treated with hydroxyzine and 14 patients treated with alimemazine were enrolled in the study. The remaining patients without antihistamines were used as control ($n = 246$ and $n = 244$, respectively).

Univariate analyses showed that the duration of the QT interval was significantly longer in patients taking hydroxyzine ($P = 0.0121$). There was, however, no statistically significant difference was observed in the duration of QT interval in patients taking alimemazine ($P = 0.8265$).

Conclusion: Our results show that administration of hydroxyzine caused a statistically significant increase in QT interval measurements. By contrast, there was no significant relationschips between the administration of alimemazine and a prolonged QT interval. This result suggests that the potential to cause QT prolongation is not a class effect. A multivariate analyse including the other risk factors for QT prolongation is needed to identify that hydroxyzine is an independent risk factor.

In psychiatric practice, these findings could have important clinical relevance for patient taking concomitant drugs that can prolong the QT interval (e.g. antipsychotic drugs and tricyclic antidepressants) and those at risk of developing cardiac arrhythmias.

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Neuroprotective effect of sequential reperfusion: involvement of mitochondrial potassium channel and monoxyde azote

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Introduction: Recently, a new concept baptized 'postconditioning' has been described to protect the heart from infarction injury, it is induced by brief sequences of ischemia reperfusion at the onset of reperfusion, thus postconditioning corresponds to a sequential reperfusion. The aim of this study was before all, to test whether this manner may protect the brain in a model of transient focal cerebral ischemia; next, to investigate the involvement of the mitochondrial potassium ATP dependent channel and the monoxide azote in this phenomenon. Finally, to elucidate the relationship between these two mediators in this model.

Methods: Rats were divided into the following groups: the control group subjected to 60 minutes middle cerebral artery occlusion followed by 24 h reperfusion. Postconditioning was performed by three cycles of 30 s reperfusion/ 30 s ischemia immediately upon reperfusion. Delayed postconditioning: for which cycles were begun after 5 minutes of reperfusion.

5Hydroxydecanoate (40 mg/kg) a specific blocker of mitochondrial potassium ATP dependent channel, and diazoxide (10 mg/kg) an agonist of this channel per se, were injected intraperitoneally at different times with or without postconditioning. Similarly, the involvement of the NO was examined by administration of N (omega) -nitro-L-arginine-methyl ester (10 mg/kg; ip.) a NO synthetases inhibitor and sodium nitroprussiate (3 mg/kg/min; iv.) a NO donor. The above drugs were used to determine the link between monoxide azote and the mitochondrial potassium ATP dependent channel. Infarct volume was determined by cresyl violet staining and measured by histomorphometric analysis.

Results: Sequential reperfusion induced a neuroprotection shown by a significant decrease of infarct size 114.35 ± 17.03 mm^3 vs. 194.79 ± 16.43 mm^3 in ischemia reperfusion ($P = 0.05$). This effect was lost once the cycles were delayed. However, neuroprotection was reproduced by diazoxide and sodium nitroprussiate and reversed by 5Hydroxydecanoate and N (omega) -nitro-L-arginine-methyl ester. Furthermore, NO is involved both upstream and downstream of mitochondrial potassium ATP dependent channel in postconditioning.

Conclusion: The onset of the reperfusion is critical for pathway salvage activation involving mitochondrial potassium ATP dependent channel and NO. Last but not least postconditioning birth renewed the reperfusion injury notion which afford a better comprehension of mechanisms responsible of infarcts damage and allow to extend therapeutic targets list or merely to ameliorate mechanically the reperfusion manner in human.

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Binding of elastin peptides to S-Gal protects the heart against ischemia/reperfusion injury by triggering the RISK pathway

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Introduction: Degradation of elastin, the longest-lived protein in humans, by elastase-type proteases is a hallmark of aging tissues and is deeply exacerbed in most cardiovascular diseases. As a consequence of such elastolysis, elastin fragments (EF) are released from the polymer and were detected in the blood circulation at a concentration range of 10^{-6}–10^{-2} mg/ml. Level of EF was found to increase significantly in the sera of patients suffering from abdominal aortic aneurysms, obliterate arteriosclerosis or ischemic heart disease. The aim of the study was to investigate the effect of elastin-derived peptides (EPs) on rat heart ischemia-mediated injury.
Methods: Langendorff hearts, after a 20-min stabilization period, were perfused for 20 min then subjected to 40 min of global ischemia and 40 min of reperfusion (I/R, Ctrl); exposed to EPs (1.32–660 nM) for 10 min at reperfusion. Hearts were treated with different inhibitors (UO126, L-NAME, Lactose and V14).The main endpoints were the mean coronary flow (MCF), the left ventricular end-diastolic pressure (LVEDP), rate-pressure product (RPP), CK release and myocardial infarct size. Results are confirmed by western-blots.
Results: EPs, elicited a beneficial influence against ischemia by accelerating the recovery rate of heart contractile parameters and by decreasing significantly creatine kinase release and heart necrosis area when measured at the onset of the reperfusion. All effects were S-Gal dependent, as being reproduced by $(VGVAPG)_3$, and as being inhibited by receptor antagonists such as lactose and V14 peptide (VVGSPSAQDEASPL). EPs interaction with S-Gal triggered NO release and activation of PI_3-kinase/Akt and Erk1,2 in human coronary endothelial cells (HCAECs and rat neonatal cardiomyocytes (RCs) . This signalling pathway as designated as RISK for reperfusion injury salvage kinase pathway, was shown to be responsible of the beneficial influence of EPs on ischemia/reperfusion injury on the basis of its inhibition by specific pharmacological inhibitiors. EP survival activity was attained at a concentration averaging that present into the blood circulation, supporting the contention that these matrikines might offer a natural protection against cardiac injury in young and adult individuals. Such protective effect might be lost with aging since we found that hearts from 24-month-old rats did not respond to EPs.
Conclusion: Our data support the contention that circulating elastin fragments or administration of such elastokines at the onset of reperfusion might reduce the infarct size. Overall these investigations attribute to elastin and its derived proteolytic fragments potent heart protective function.

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Chronic pravastatin therapy increases outgrowth endothelial progenitor cells in patients with stable coronary artery disease

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Introduction: Previous studies have shown that early and outgrowth endothelial progenitor cells (EPCs) can be detected in the peripheral blood. Furthermore, statin therapy can increase EPC mobilization into the peripheral blood. However there are no data concerning the long-term effects of statin treatment on this phenomenon. The aim of this study was to investigate whether EPCs can be detected and characterized in patients with at least 4 weeks statin therapy.
Methods: Two groups of patients were matched for sex, age and treatment. The statin (+) group ($n = 7$) included patients with stable coronary artery disease (CAD) treated with pravastatin 40 mg for at least 4 weeks.
The statin (-) group ($n = 7$) included patients with stable angina without statin therapy. Mononuclear cells from all patients were assessed for progenitor cell (CD34, CD34/CD144 and CD34/CD117) and endothelial phenotype (VEGF-R2, CD31) by flow cytometry and were cultured to determine the number and the type of EPCs.
Results: The results show that there was a significantly higher number of circulating progenitor cells in the statin (+) group. The culture showed that circulating early EPCs were CD146+ and CD45+ and were also significantly higher in the statin (−) group. Surprisingly, circulating outgrowth EPCs were only found in the statin (−) group. Phenotyping of cultured cells showed that cells from outgrowth EPC colonies clearly had an endothelial phenotype CD31+, VEGF-R2+, CD34+ in contrast to cells from early EPC colonies (VEGF-R2low, CD34−, CD45+).
Conclusion: These results show that, (i) EPCs can be found in the peripheral blood of CAD patients treated up to 4 weeks with 40 mg of pravastatin; (ii) long-term statin therapy raises EPC levels by increasing outgrowth EPC population without affecting early population levels; and (iii) the phenotype of early EPC is different from that of outgrowth EPCs suggesting that early EPCs are not endothelial but hematopoietic in origin.

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Critical role of angiotensin converting enzyme level in acute myocardial ischemia-reperfusion injury revealed by gene titration in the mouse

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Introduction: A common polymorphism of the angiotensin converting enzyme (ACE) gene has been shown in man to be associated with differences in circulating ACE levels that confer a differential risk for cardiovascular diseases. To determine whether ACE level could influence infarct size in the setting of *in vivo* experimental myocardial ischemia-reperfusion injury, we used genetically modified mice bearing one, two or three functional copies of ACE gene, having ACE levels ranging from 62% to 154% of wild-type mice and no difference in blood pressure.
Methods: Adult male mice were submitted to ischemia-reperfusion injury (30 min coronary occlusion followed by 180 min reperfusion). Area at risk (AR) and myocardial infarct size (IS) were determined by planimetry and IS/AR ratios (%) were calculated. In a first set of experiments, we studied the effect of ischemia-reperfusion injury in one-copy ($ACE^{+/-}$: $n = 8$), two-copy ($ACE^{+/+}$: $n = 12$) or three-copy (ACE^{3+}: $n = 8$) mice in basal conditions or after ischemic preconditioning (IPC, three cycles of 3 min occlusion followed by 5 min reperfusion; IPC-$ACE^{+/-}$: $n = 8$; IPC-$ACE^{+/+}$: $n = 14$; IPC-ACE^{3+}: $n = 9$). In a second set of experiments, wild type mice were treated by an ACE inhibitor, ramiprilat (50 μg/kg, iv, $n = 13$) or saline ($n = 19$) 5 min before reperfusion, and were subjected to the same ischemia-reperfusion injury.
Results: Infarct size of mice having one-copy of the ACE gene (26.9 ± 3.0%) was significantly lower (−30%, $P = 0.05$) relative to that of the normal (two-copy) mice (38.4 ± 2.6%). The cardioprotection afforded by ramiprilat in normal mice (−29%, $P < 0.01$) was the same as in untreated one-copy mice. In three-copy mice, infarct size (40.6 ± 3.9%) was not different from two-copy mice. Ischemic preconditioning reduced infarct size in the normal mice from 38.4 ± 2.6% to 14.1 ± 1.1% (-63%, $P < 0.001$), and in the one-copy mice from 26.9 ± 3.0% to 13.4 ± 1.5% (-49%, $P < 0.05$), but the cardioprotective effect of ischemic preconditioning was completely abolished in the three-copy mice (39.7 ± 3.5%).
Conclusion: We conclude that a modest genetic decrease in ACE activity level reduces, like ACE inhibition by ramiprilat, ischemia reperfusion injury while a modest increase in ACE activity level does not aggravate infarct size but suppresses the cardioprotection afforded by ischemic preconditioning. As the effect of ACE gene duplication is the same as that of tissue kallikrein gene inactivation, our data support the hypothesis that kinins play an important role in ischemic preconditioning. Furthermore, the results provide a mechanistic hypothesis and document a causality link for the proposal association between the genetic polymorphism of ACE and the risk of myocardial infarction in man.

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Comparative study of the signaling pathways of the wild-type and deleted-form of human α2B-adrenergic receptor, from the membrane to the nucleus

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Introduction: A common polymorphic variant of the human α2B-adrenoceptor (α2B-AR) consists in the deletion in the third intracellular loop of three glutamic acids located within a stretch of acidic residues thought to promote agonist-induced receptor phosphorylation by G protein-coupled receptor kinases. The deleted variant (Del α2B-AR) was found to exhibit decreased phosphorylation and impaired desensitization compared to the wild-type (WT α2B-AR). The present study was designed to compare signalling activity of the WT and the Del α2B-AR.
Methods: Pig kidney LLC-PK1 cells were stably transfected to express similar levels of each HA-tagged α2B-AR variant. Receptor phosphorylation, β-arrestin recruitment, phosphorylation of Erk1/2, Akt and IKKα/β were assessed by western blotting. NF-κB activation was followed up using a luciferase reporter gene construct.
Results: In response to agonist exposure, the phosphorylation extent of the Del α2B-AR was decreased compared to that of the WT. Furthermore, depressed phosphorylation of the Del α2B-AR resulted in a prolonged coupling with G-proteins and a slower kinetics of β-arrestin recruitment. In both cell types, α2-agonist UK14304 induced rapid and long-lasting increase in the phosphorylation of Erk1/2. This effect was partially inhibited by MMP inhibitors, heparin, and tyrphostin AG1478 and persisted upon EGFR desensitization, indicating that it is triggered by EGFR-dependent and EGFR–independent mechanisms. Phosphorylation of Erk1/2 and Akt following α2-agonist exposure was strongly attenuated by the inhibitor of MEK1/2 (PD98059), of PI3-Kinase (LY240092) and of Src (PP1). UK14304 treatment caused also IKKα/β phosphorylation and activation of NF-κB. LY240092 inhibited IKKα/β phosphorylation and NF-κB activation by both forms of α2B-AR. In contrast, PD98059 was efficient in cells expressing the Del, but not the WT α2B-AR.
Conclusion: Altogether, these data demonstrate that the WT and the deleted form of α2B-AR activate MAPK and Akt by similar pathways in LLC-PK1 cells. However, MAPK and Akt are differently involved in NF-κB activation triggered by the two α2B-AR variants.

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The peripheral benzodiazepine receptor is a new target for protection of the myocardium against ischemia-reperfusion injuries

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Introduction: The peripheral benzodiazepine receptor is located on the outer membrane of mitochondria where it mediates the transport of cholesterol. This receptor is highly concentrated in the organs synthesizing steroid hormones but it is also present in the heart where its function remains unknown. As this receptor is closely associated with the components of the mitochondrial permeability transition pore, a multimeric structure involved in myocardial ischemia-reperfusion injury, we hypothesized that interaction with this receptor might mediate cardioprotection. In this study we (i) assessed the potential cardioprotective effect of chlorodiazepam, a specific peripheral benzodiazepine receptor ligand in an *in vivo* model of ischemia-reperfusion and (ii) determined the mitochondrial mechanism associated with this effect.
Methods: Anesthetized male Wistar rats were submitted to 35 min occlusion of the left coronary artery followed by different times of reperfusion. After sacrifice, the hearts were removed, mitochondria were isolated and the following parameters were evaluated: infarct size by tetrazolium staining, apoptosis by dUTP tunel staining, cytochrome c release by western blot analysis, mitochondrial respiration and mitochondrial transition pore opening. Chlordiazepam (0.5–10 mg/kg) was given i.v. 10 min before ischemia.
Results: Chlorodiazepam reduced infarct size expressed as the percentage of the risk area at 5 [17 ± 2% ($n = 6$)] and 10 mg/kg [11 ± 1% ($n = 6$)] as compared with control [31 ± 3% ($n = 9$) all $P < 0.05$]. This cardioprotective effect was associated with a reduction in apoptosis as demonstrated by a decrease in the number of tunel

positive staining cardiomyocytes [from 22 ± 9 to 6 ± 2% (n = 6) $P < 0.05$]. Chlorodiazepam also improved the ability of mitochondria to synthesizeTP as attested by the increase in the respiratory control ratio. These effects were due to a limitation of the permeability of the mitochondrial membrane as chlorodiazepam inhibited the release of cytochrome c measured 1 h after reperfusion. However, these effects were independent of a direct inhibition of the mitochondrial permeability transition pore opening as the drug did not decrease the sensitivity of mitochondria to pore opening evaluated by their capacity to retain calcium.
Conclusion: Taken together these data demonstrate that the peripheral benzodiazepine receptor is a relevant target to protect the myocardium against ischemia-reperfusion induced injuries. This protection could be mediated by a limitation of the permeability of the outer mitochondrial membrane but is independent from a direct inhibition of the mitochondrial transition pore opening.

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Prevention of liver injury by BHDP, a sigma1 ligand, in two rat models of liver ischemia

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Introduction: Ischemia-reperfusion damages are encountered in all clinical circumstances where there is a reduction or an interruption of blood flow followed by reperfusion. Ischemia leads to a decrease of both oxygen and nutriment supply and therefore to an impairment of the cellular metabolism. Over more, by causing a massive calcium overload, an oxidative stress and a decrease in ATP synthesis reperfusion can induce irreversible damage on the cell. Different methods were used to prevent or to reduce the harmful consequences of ischaemia-reperfusion.
Methods: In the present study, we have investigated the antiischemic properties of a compound having high affinity and selectivity for sigma$_1$ receptors, N-benzyl-N'- (2-hydroxy-3,4-dimethoxybenzyl) -piperazine (BHDP), in two different models of ischemia. The first model was an experimental model of rat liver normothermic ischemia-reperfusion. Rats were pretreated with different doses of BHDP (0.5, 2.5 or 10 mg/kg/day) or solvent alone and subjected to 90 min normothermic ischemia followed by either 30 or 120 min reperfusion. The second model was an hypothermic model of ischemia where livers were incubated for 24 h at 4°Cin a preservation solution in the absence or in the presence of various BHDP concentrations (0.5, 2.5 or 10 μg/ml). At the end of the experiments, livers were removed and mitochondria were isolated. Liver and mitochondrial functions were assessed.
Results: These different ischemic conditions induced huge alterations of hepatocyte functions, namely, membrane leakage of alanine aminotransferase and aspartate aminotransferase, decreased metabolic capacities evaluated by the ability of the liver to transform lidocaine, alterations of mitochondrial functions characterized by a decrease in ATP synthesis and the appearance of histological damages. Pretreatment of rats with BHDP alleviated these deleterious ischemia-reperfusion effects at both the cellular and mitochondrial levels in a dose-dependent manner. The protection of mitochondrial functions was almost complete at a dosage of 10 mg/kg/day during normothermic ischemia and at a concentration of 10 μg/ml in the liquid of preservation during hypothermic ischemia. In addition, BHDP reduce significantly the histological damages and improve liver metabolic capacities.
Conclusion: These data demonstrate that BHDP protects liver against the deleterious effects of ischemia-reperfusion and suggest that sigma$_1$ receptors play an important role in the protective effect.

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Arginase inhibition decreases blood pressure and improves vascular reactivity in hypertensive spontaneously hypertensive rats

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Introduction: Recent studies reported an upregulation of vascular arginase in hypertensive animals. Arginase reciprocally regulates NO levels in endothelial cells by competing with NO synthase for the substrate L-arginine. Given the well-established role of endothelial dysfunction in pathophysiology of hypertension, arginase appeared as a novel target for therapy in hypertension. The aim of this study was to determine the effect of the selective arginase inhibitor Nω-hydroxy-nor-L-arginine (nor-NOHA) on blood pressure as well as vascular reactivity and remodeling.
Methods: Ten-week-old male SHR were treated with nor-NOHA (10 or 40 mg/kg/d, ip) for 3 weeks. A group of untreated SHR and normotensive Wistar Kyoto (WKY) rats served as controls. Systolic blood pressure (SBP) was measured in conscious rats by the tail-cuff method. The response of Ach was measured in aortic rings whereas the flow-dependent vasodilation, myogenic tone and media/lumen (M/L) ratio were determined in 3rd order mesenteric artery. Tissue arginase activity and circulating levels of urea and glucose were determined at the end of the treatment.
Results: SBP was kept close to the pretreatment value with both doses of Nor-NOHA. As compared to WKY rats, untreated SHR exhibited decreased Ach-dependent vasodilation and flow-dependent vasodilation and increased myogenic tone (P = 0.05). Treatment with nor-NOHA dose-dependently restored these parameters in SHR. By contrast, hypertension-induced vascular remodeling was not changed after nor-NOHA treatment. The two doses of Nor-NOHA resulted in a 30–40% decrease in liver and kidney arginase activity ($P < 0.05$). Of note, arginase inhibition did not affect the circulating levels of urea and glucose.
Conclusion: Our data demonstrated that administration of a selective arginase inhibitor for 3 weeks in hypertensive SHR stabilized blood pressure to pretreatment value and improved vascular reactivity of both conductance and resistance arteries. The good tolerance of arginase inhibition in SHR encourages to investigate the effect of long-term administration of arginase inhibitor in SHR.

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Heart rate reduction with ivabradine protects against ventricular fibrillation during acute myocardial ischemia in pigs

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Introduction: Sudden death due to ischemic ventricular fibrillation (VF) is often facilitated by tachycardia. Our goal was to evaluate the impact of heart-rate (HR) reduction with ivabradine (IVA), a specific inhibitor of the cardiac pacemaker current I_f, on ventricular fibrillation threshold (VFT) during acute myocardial ischemia (MI).
Methods: MI was obtained in anesthetized open-chest pigs by total and brief occlusion (1 min) of the left anterior descending artery, on two occasions at 15-min interval at baseline and then on two occasions at 15-min interval following the I.V. Administration of either saline (n = 10) or IVA 0.5 mg/kg (n = 10). HR, monophasic action potential duration (dMAP), LV dP/dt and VFT were monitored during each MI. VF was triggered by trains of electrical stimuli of increasing intensity at fixed rate (200 and 130 impulses/min in saline and IVA-treated group, respectively). At the end, ischemic area was measured on heart sections stained for mitochondrial succino-dehydrogenase activity using nitroblue tetrazolium.
Results: All parameters were unchanged on all four MI occasions in the saline group. IVA reduced heart rate by 32%, significantly increased VFT by 2.3-fold vs. Saline and prevented the shortening of dMAP induced by ischemia (saline: –13 ± 10 ms; IVA: +4 ± 10 ms) without effect on peak LV dP/dt. An inverse correlation between heart rate and VFT (R = 0.49, P = 0.001) was also found. Histoenzymology showed a significant decrease of the ischemic area.

	Heart rate (bpm)	Peak LV dP/dt (mmHg/s)	Ventricular fibrillation threshold (mA)	Ischemic area (% of LV area)
Saline (n = 10)	108 ± 6	1028 ± 92	3.2 ± 0.4	37.6 ± 3.2
Ivabradine (n = 10)	73 ± 6 ***	1023 ± 61	7.2 ± 0.6***	26.4 ± 2.9***

*P = 0.05

Conclusion: HR reduction with IVA during acute MI, in the absence of other hemodynamic effects, protects against VF and decreases myocardial damage. These data suggest that specific HR reduction with IVA may offer a new therapeutic approach in the prevention of sudden death due to ischemic VF.

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Does norepinephrine worsens functional mitral regurgitation?

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Introduction: In literature, there are concerns regarding the use of vasopressor such as norepinephrine in the setting of functional MR. However, this caution is still debated.
Methods: Four patients presenting with clinical signs of shock requiring norepinephrine infusion and having MR and Left ventricular systolic dysfunction due to ischemic and non ischemic cardiopathy were studied. The Doppler proximal flow convergence method was used to quantify MR both before and after norepinephrine administration.
Results: Before norepinephrine infusion, mean systolic blood pressure and mean LV ejection fraction were 83 ± 7 mmHg and 25 ± 7% respectively. After norepinephrine administration, mean systolic blood pressure increased to 113 ± 6 and LV EF was measured at 23 ± 7%. The use of norepinephrine was associated with a significant reduction in MR severity. Mean mitral effective regurgitant orifice area and regurgitant volume decreased from 10 ± 4 mm^2 and 9 ± 4 mL to 8 ± 3 mm^2 and 7 ± 3 mL respectively.
Conclusion: In conclusion, norepinephrine infusion does not necessarily aggravate secondary MR. Further studies are needed to determine how norepinephrine modifies the balance between mitral closing and tethering valve forces.

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Roles of the 5-HT2B receptor in cardiac hypertrophy: a link with oxidative stress?

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Introduction: Oxidative stress generated by the activation of NAD (P) H oxidase is a main contributor to left ventricular hypertrophy (LVH). The aim of this study was to verify if the blockade of 5-HT$_{2B}$ receptors (5-HT$_{2B}$R) could reduce LVH induced by angiotensin II (AGII) and isoproterenol (ISO) through a reduction of myocardial oxidative stress.
Methods: Adult 129/SvPAS male mice were infused either with saline, ISO (30 mg/g/day, 5 days), ISO + SB215505 (a 5-HT$_{2B}$R antagonist, 1 mg/kg/day), AGII (200 ng/kg/min, 14 days) or AGII + SB215505. After perfusions, mice were recorded for blood pressure and heart rate, cardiac anatomy and function. Superoxide anion (SA) production was measured in the abdominal aorta and right and left ventricles (LV) by the lucigenin method in basal and stimulated (100 mM NAD (P) H) conditions. LV p47-phox subunit expression of the NAD (P) H oxidase was assessed by western blots.
Results: ISO induced a LVH (6.3 ± 0.3 mg/g, n = 9 vs. 2.9 ± 0.2 mg/g, n = 8 in saline-treated animals, $P < 0.05$) which was associated with an increase in NAD (P) H stimulated SA production in the LV (35 ± 3 cpm/mg, n = 9 vs. 24 ± 1 cpm/mg, n = 8 in controls, $P < 0.05$). No effect was seen in the right ventricle and the aorta. SB215505 prevented LVH (3.2 ± 0.2 mg/g, n = 8, $P > 0.05$ vs. controls) and NAD (P) H stimulated SA production (24 ± 2 cpm/mg, n = 8, $P > 0.05$ vs. controls). AGII induced a LVH (4.7 ± 0.1 mg/g, n = 8 vs. 3.1 ± 0.1 mg/g, n = 8 in controls, $P < 0.05$) which was accompanied by an increase in blood pressure (154 ± 5 mmHg vs. 117 ± 7 mmHg in controls, $P < 0.05$) and of LV basal (+32% vs. controls, P = 0.05) and stimulated (+84% vs. controls, P = 0.05) SA production. The production of SA was increased in the aorta (114 ± 9 cpm/mg vs. 62 ± 6 cpm/mg in controls, P = 0.05). SB215505 completely prevented the LVH (3.4 ± 0.2 mg/g, n = 8, $P > 0.05$ vs. controls) and basal as well as stimulated LV oxidative stress but was without effect on blood pressure and vascular oxidative stress. ISO and AGII both induced the LV overexpression of p47-phox and SB215505 was without effect on these inductions.
Conclusion: In conclusion, ISO and AGII cardiac receptors stimulations induce a LVH and an increase of myocardial oxidative stress which are both reduced by 5-HT$_{2B}$R blockade. The attenuation of the oxidative stress although mediated by a decrease in NAD (P) H oxidase activity is nevertheless not mediated by a reduction in the p47-phox overexpression.

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The release of troponin I and the dynamic of circulating endothelial and hematopoietic progenitors in patients with acute coronary

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Introduction: Different types of circulating progenitor cells can be detected in the peripheral blood of healthy subjects: hematopoietic progenitors (HPs), early and late endothelial progenitors (EPCs). Early EPCs were described with a limited proliferation capacity whereas late EPCs possessed a strong outgrowth. EPCs can be also mobilised into the peripheral blood in the setting of the acute myocardial infarction. The aim of this study was to compare the release of troponin I (TnI) to the dynamics, magnitude and the types of progenitor mobilization in patients with acute coronary syndrome.

Methods: Patients with acute coronary syndrome (ACS) (n = 24) were enrolled in this study. Peripheral blood was drown in admission (day 0) and after 7 days. The release of TnI was quantified 12 hours after admission. The patients were sorted in three groups according the rate of TnI released into the blood. The cells were cultured in specific conditions. The endothelial (early and late EPC) and hematopoietic (CFU-GEMM, GM and BFU-e) origin of the colonies were confirmed *in vitro* by phenotypic study. The number of colonies was also determined.

Results: The table shows the clonal efficiency of endothelial progenitors. Only early EPCs and BFU-e at day 0 were significantly higher in patients with the rate of TnI < 25 µg/L compared to the other patient groups. Furthermore, the number of early EPCs at day 0 decreased when the rate of TnI increased (α (P = 0.03, R2 = 0.38). At day 7 there were no significant differences between the number of EPCs and HPs and the rate of troponin release.

Conclusion: The circulating early EPCs were disturbed in patients with ACS according to the rate of TnI. Thus, the number of endothelial progenitors can be related to the myocardial injury. This study will be presented with more patients.

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Effects of losartan in an experimental model of metabolic syndrome

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Introduction: A large body of experimental and clinical evidence indicates that some AT_1 receptor antagonists may have beneficial metabolic effects in addition to their well-known cardiovascular actions. Whether or not these metabolic effects are related to additional PPARγ agonist activity of some AT_1 antagonists is still under debate. Therefore, the aim of the present study was to check the cardiovascular and metabolic effects of losartan lacking any PPAR agonist activity in a suitable experimental model of metabolic syndrome, namely SHHF rats (spontaneously hypertensive, heart failure). These rats exhibit obesity, hypertension, dyslipidemia and glucose intolerance. They lack leptin receptors.

Methods: Losartan was delivered in the drinking water (10 mg/kg/day during 3 months) to 12-week-old male SHHF rats. Cardiovascular and metabolic parameters were measured at the end of the treatment and compared to those of untreated SHHF rats at the same age. Intravenous glucose tolerance tests (IVGTT) were also performed. Total cholesterol, LDL, HDL, triglycerides and glucose were measured on plasma samples (0.5 ml) taken from caudal veins. Blood pressure was measured (right femoral artery) under pentobarbital anaesthesia (60 mg/kg, ip). Mean values ± SEM are presented. P values <0.05 were considered significant. Unpaired Student's t-tests were used for intergroup comparisons.

Results: Effects of a 3-month treatment are shown in the following table.

	Untreated SHHF (n = 5)	Losartan treated SHHF (n = 5)
Mean blood pressure (mmHg)	159 ± 3	102 ± 4.3 *
Body weight (g)	552 ± 11.7	584 ± 10.9
Glucose (mmol/l)	9.58 ± 1.08	10.35 ± 0.74
Cholesterol (mmol/l)	3.97 ± 0.39	3.96 ± 0.16
HDL (mmol/l)	1.51 ± 0.08	2.38 ± 0.13 *
LDL (mmol/l)	0.61 ± 0.11	0.59 ± 0.044
Triglycerides (mmol/l)	5.69 ± 0.57	6.74 ± 0.37
Free fatty acids (mmol/l)	1.74 ± 0.2	1.5 ± 0.075

Glucose tolerance remained impaired in losartan treated animals.

Conclusion: Our study showed that, at an antihypertensive dose, losartan exhibited only one significant metabolic effect, an increase of HDL. This effect is of course interesting but partial.

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Droperidol and ondansetron induced QT-interval prolongation: a clinical drug interaction study

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Introduction: Droperidol and 5-HT3 antagonists, such as ondansetron, are the most effective antiemetics used in the treatment/prophylaxis of postoperative nausea and vomiting (PONV). They are frequently combined in patients at high risk. These drugs have been shown to prolong the QT-interval (1) which, for droperidol, led the FDA to a label warning. The goal of this study was to compare the electrocardiographic effects of these two drugs and to assess the effect of the combination on QT-interval prolongation.

Methods: Sixteen healthy volunteers participated in this prospective randomized, double blind, four-way cross-over study. Subjects received a placebo injection, droperidol (DRO 1 mg I.V.), ondansetron (OND 4 mg I.V.) alone and in combination in a random order. Continuous 12-leads digital ECG were recorded. Blood samples were obtained at the end of drug administration and after 2, 4, 6, 10, 20, 30, 45, 60, 120, 240, 420 and 600 minutes and analyzed using LC/MS method. QT-interval was corrected using Fridericia's formula. A time-matched placebo and baseline subtracted approach was used to calculate QTc prolongation at each time point.

Results: DRO and OND administered alone and in combination significantly prolonged QTc. The mean (±SD) maximal QTc prolongation was 25 ± 11 ms after DRO and 19 ± 9 ms after OND (DRO vs. OND P = 0.07). Maximal QTc prolongation during DRO and OND combination was 25 ± 10 ms and was not significantly different compared to DRO (P = 0.95) and OND (P = 0.07). Compared to placebo, neither DRO nor OND used alone or in combination modified heart rate (P = 0.09). Pharmacokinetic analyses showed no drug interaction. The ratio of log transformed AUCs of drug administered in combination/alone were 0.99 (IC90: 0.85–1.15) and 0.97 (IC90: 0.88–1.07) for DRO and OND respectively.

Conclusion: This study confirms the QTc lengthening induced by low doses of DRO and OND. Although there was a trend toward greater QTc prolongation after DRO than after OND, the combination of the two drugs did not induce greater QTc prolongation compared to DRO alone. These results confirm electrophysiological interaction data (2) showing less potent prolongation of repolarisation with OND at therapeutic concentrations. Combination of these potent antiemetics has not additive or synergistic cardiac effects and is therefore not expected to increase the proarrhythmic potential of their combined use.

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Genetic susceptibility to hypertension and chronic intermittent hypoxia as a rat model of obstructive sleep apnoea syndrome

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Introduction: Obstructive sleep apnoea syndrome (OSA) is associated with an increased risk in developing cardiovascular pathologies such as hypertension. The current treatment known to prevent its deleterious consequences is restrictive. Research is thus focused on elucidating the mechanisms involved in OSA in order to find new therapeutic strategies. The aim of this work was to characterise a model designed to mimic the cardiovascular consequences of OSA by combining intermittent hypoxia (IH) with a genetic susceptibility to hypertension.

Methods: Nine-week old spontaneous hypertensive (SHR) (n = 27) and control Wistar Kyoto (WKY) (n = 16) rats were exposed to IH (5% FiO_2) or to normoxia (N), 8 hours/day, during 14 days. Bodyweight and systolic arterial pressure (SAP), recorded by plethysmography, were measured at days 1, 8 and 15 (D1, D8, D15) of exposure. Blood pressure was also assessed at D15 following arterial catheterisation. The Langendorff perfusion mode was used at constant pressure to assess infarct size following an ischemia-reperfusion (I/R) and at constant flow to study the coronary vasoconstrictive response to bolus injections of endothelin-1 (ET-1: 10^{-9} to 3.10^{-6} M).

Results: Body weight was greater in WKY than in SHR and in normoxic than in hypoxic animals. Hematocrit was significantly increased by hypoxia, particularly in the SHR group. SAP values of SHR were higher and were increased by IH at D15 (222 ± 6 compared to 191 ± 6 and 194 ± 6 mmHg at D1 and D8, respectively, $P \leq 0.05$). Catheterisation measures confirmed these results. IH aggravated infarct size in both SHR and WKY (34.6 ± 3.9% and 34.7 ± 4.6%, respectively) compared to normoxic rats (25.2 ± 4.6% and 26.4 ± 2.9%, respectively, $P \leq 0.05$). In the SHR IH group, there was a significantly greater increase in coronary perfusion pressure (116.3 ± 6.3 mmHg compared to 80.5 ± 8.0, 77.4 ± 8.9 and 83.3 ± 8.4 mmHg in SHR N, WKY IH and WKY N groups, respectively, $P \leq 0.05$) and in left ventricular end-diastolic pressure in response to ET-1 (3.10^{-6} M).

Conclusion: The combination of IH with genetic susceptibility to hypertension accelerates the development of hypertension, alters the metabolism and increases the coronary and myocardial response to ET-1. Exposure to IH also resulted in an aggravation of myocardial infarction after I/R. This model was thus able to reproduce OSA with a major role for IH in the development of its associated pathologies. The follow-up to this study will be to determine the cellular mechanisms involved with particular emphasis on the role of endothelin-1 and its receptors.

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Candesartan on the top of standard therapy decrease sympathetic activity in patients with advanced heart failure: pilot study

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Introduction: The CHARM trial has demonstrated that candesartan can significantly reduces all-cause mortality, cardiovascular death, and heart failure hospitalizations in patients with CHF and LVEF below 40% when added to standard therapies including ACE inhibitors, beta-blockers, and an aldosterone antagonist. We sought to assess if this benefit could be explained by a reduction in the sympathetic tone of heart failure patient.

Methods: In a group of randomly and prospectively selected advanced heart failure patients we assessed the sympathetic tone through recording of muscle sympathetic nerve activity. Recordings were done without knowing the medical regimen of the patients. Clinical, biological and morphological data were collected. We compared the sympathetic nerve activity in the group of patients with or without candesartan.

Results: Among 11 patients with advanced heart failure, five received candesartan at a mean dose of 4.8 mg. Patients in the candesartan group had the same age (59 ± 5-years old), their functional status was not different (mean peak VO2: 13.8 ml/kg/min). The muscle sympathetic nerve activity was lower in the candesartan group (51 ± 5 burst/min vs. 62 ± 4, P = 0.05) and this difference was maintained even after adjustment for heart rate. Patients with candesartan had higher haemoglobin level (14.3 g/dl vs. 12.1 g/dl, P < 0.05) and lower BNP level (60.3 ± 145 vs. 884 ± 101 pg/ml, P = 0.05) despite same ejection fraction and functional status.

Conclusion: This cognitive study shows that the benefit of candesartan add-on therapy could be related to a decrease in the sympathetic tone. Hypothetical mechanisms are multiple among them cross talk between renin and sympathetic system or effect of candesartan on Hemoglobin, a well known prognostic factor in heart failure.

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Temporal and spatial changes in free iron levels after brain ischemia in rats

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Introduction: Whereas free iron is thought to be a potential target for therapeutic intervention in stroke, the time course of iron metabolism disruption have never been explored after ischemia. In the present study free iron levels and expression of

ferritin (Ft), the main iron storage protein, were measured after a permanent focal ischemia in rats. We also evaluated the potential benefits of a delayed administration of dipyridyl (DP), a liposoluble iron chelator whose preischemic administration just before and after brain ischemia was already reported to reduce blood brain barrier (BBB) damage.
Methods: A cortical infarct was unilaterally induced in rats using the photothrombotic ischemic stroke model. Measurements of ultrafiltrable iron (atomic absorption spectrometry) and Ft levels (Western blotting) were performed in control and ischemic rats. Cortical tissue samples corresponding to the ischemic core (IC) and penumbra (P) were collected at times 1, 4 h and 1, 3 and 8 days post-ischemia (n = 5–8 per group). DP or vehicle was administered at 4 h post-ischemia and damage to the BBB was quantified by the Evans blue method (n = 8 per group).
Results: Disruption of iron metabolism was confined to cortex ipsilateral to the lesion. As compared to contralateral values, high free iron levels (nmol/g of protein) were observed in IC and P but only at time 1 h post-ischemia (57.6 ± 16.2 vs. 176.4 ± 46.2 for IC and 55.7 vs. 155.9 ± 29.9 for P). Consistent with the rapid return to normal values of free iron level, DP did not reduce BBB damage. Finally, ischemia resulted in a delayed and sustained increase in Ft expression. Ft upregulation was earlier for L-Ft- than H-Ft [(1 vs. 3 day) and more intense for H-Ft than L-F (6- vs. 4-fold) at day 8 as compared to control values].
Conclusion: Our results report an early and transient accumulation of free iron as well as a delayed and sustained Ft upregulation after brain ischemia. They suggest that treatment with iron chelator has to be started as soon as possible after stroke onset, at least if the aim of the treatment is to inactivate free iron excess.

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Spatiotemporal brain NOS II et arginase expression following focal cerebral ischemia in rats

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Introduction: Brain expresses arginase 1 and 2, enzymes that transforms L-arginine to urea and L-ornithine, a precursor of polyamines synthesis. Polyamines are recognized to be essential for regenerative processes and may be involved in repair and functional restoration after a brain lesion. Moreover, lesion to the brain results in induction of NOS II, an enzyme that competes with arginase for metabolizing L-arginine into nitric oxide. Therefore, polyamine synthesis is not only dependent on arginase but also on NOS II expression. In the present study, we investigated for the first time the effect of brain ischemia on arginase expression and compared arginase and NOS II expression in term of spatial and temporal changes.
Methods: A focal brain ischemia resulting in a reproducible and well delimitated cortical infarct was induced in rats by the photothrombotic ischemic stroke model. Cortical samples was collected at the infarct level and in the areas surrounding the infarct at 1, 3 and 8 days after ischemia. Corresponding regions were also collected in control rats. Expression of NOS II and the both isoforms of arginase was assessed using Western blotting analysis. Immunochemistry against arginase 1 was also performed on brain section.
Results: Control rats exhibited high expression of arginase 1 and 2, whereas NOS II was not detectable. Immunostaining revealed a sustained arginase 1 labelling in neurons in both perikaryon and neurites. As compared to control values, arginase expression was not modified by ischemia whatever the post-ischemia time and the brain area location. In contrast, ischemia resulted in NOS II induction which was more intense at the infarct level than outside the infarct. Expression of NOS II peaked at 3 days and then progressively decreased.
Conclusion: Our results report different spatial and temporal profile of expression between arginase and NOS II after brain ischemia, suggesting the lack of competition of the two enzymes for catabolizing L-arginine.

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Effect of a neutral endopeptidase inhibitor, thiorphan on myocardial ischemia-reperfusion injury

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Introduction: Myocardial ischemia-reperfusion has been shown to be associated with lethal reperfusion injury and cardiac arrythmias. Kinins are generally considered to be cardioprotective and ACE inhibitors which preserve bradykinin from degradation, have been reported to reduce myocardial ischemia-reperfusion injury through B2 receptor activation. Neutral endopeptidase (NEP) is a non selective metalloprotease which degrades natriuretic peptides and bradykinin. In this context, the aim of this study was to determine whether inhibition of NEP by thiorphan is able to reduce infarct size in an *in vivo* model of myocardial ischemia-reperfusion in the mouse.
Methods: In a first set of experiments, to determine a dose of thiorphan which inhibits NEP selectively, adult male C57BL/6 mice were divided into four groups (n = 5/group) receiving an infusion (17 µL/h/mouse) of saline or thiorphan (1, 5, or 15 µg/kg/min). Maximal blood pressure changes triggered by angiotensin I (100 ng/kg, 300 ng/kg) and bradykinin (300 ng/kg, 1000 ng/kg) as 5 µL/g iv bolus, were measured. In a second set of experiments, mice were submitted to an ischemia-reperfusion injury (30 min coronary occlusion followed by 180 min reperfusion) and were randomly infused with saline (n = 10) or thiorphan (5 µg/kg/min, n = 10). The infusion was started 20 min before reperfusion and maintained up to the end of reperfusion. Area at risk (AR) and myocardial infarct size (IS) were determined by computerized video planimetry after Evans blue and triphenyltetrazolium chloride staining respectively. IS/AR ratio (%) was calculated.
Results: Thiorphan dose dependently increased the extent of hypotension by bradykinin: at 1 µg/kg/min this effect was modest and not significant vs. bradykinin 300 ng/kg. At 5 µg/kg/min, pressor responses to bradykinin were significantly enhanced, whereas those to angiotensin I were unchanged. At 15 µg/kg/min, thiorphan lowered blood pressure and inhibited pressor responses to angiotensin I by approximately 30%. At the selective non hypotensive dose of 5 µg/kg/min, thiorphan reduced infarct size (25.7 ± 3.9 vs. 39.2 ± 2.4, $P < 0.01$). Areas at risk did not differ between control (32.5 ± 2.3%) and treated groups (28.3 ± 1.7, NS).
Conclusion: The results demonstrate for the first time in mice, that selective NEP inhibition, as ACE inhibition with ramiprilat, provides cardioprotection against myocardial ischemia-reperfusion. They suggest that kinins might be involved in the mechanism of this protective effect, a hypothesis that needs to be further tested in mice genetically deficient in the kallikrein-kinin system.

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Hypoxia differentially regulates angiogenic properties of human endothelial progenitor cells in culture

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Introduction: An increasing number of studies provide evidence that hypoxic preconditioning enhance neovascularization efficacy of human endothelial progenitor cells (EPCs) transplanted into the ischemied hindlimb of rats but the exact effects of hypoxia on EPCs phenotype and vesiculation are not fully characterized. We have recently shown that hypoxia increased the number of microparticles released by EPCs. We now present here preliminary results showing the effects of hypoxia on the angiogenic properties of EPCs.
Methods: Human EPCs were incubated for 24 hours in growth factor free-medium with 5% of fetal bovin serum, in presence of cobalt chloride (CoCl2, 150 µM: chemical hypoxia) or in oxygen free-atmosphere with bubbled-medium (5%CO_2, 10%H_2/N_2: drastic hypoxia) or in 1.5% oxygen-atmosphere without bubbled-medium (1.5% O2, 5% CO_2 and 94.5% N_2: soft hypoxia) or in normoxic conditions (21% O_2, 5% CO_2 and 74% N_2). Cell viability was estimated by LDH release and MTT reduction. Angiogenic properties were estimated by VEGF release with enzyme-linked immunosorbent assay (ELISA) at the end of hypoxia, proliferation (wound-healing test) and formation of complex three-dimensional capillary-like structures on matrigel 9 hours after the end of hypoxia.
Results: EPCs viability (expressed as % of normoxic conditions) was significantly altered by chemical (MTT: 82 ± 7% and LDH: 8 ± 5%) and drastic hypoxia (MTT: 64 ± 10% and LDH: 23 ± 19%) whereas soft hypoxia seemed to protect EPCs (105 ± 11% and LDH: -4 ± 8%). Surprinsingly, number of cells estimated by counting was identical in all conditions (normoxia: 354 ± 153, chemical hypoxia: 330 ± 133, drastic hypoxia: 334 ± 95, soft hypoxia: 333 ± 147.10^3). Compared to normoxic conditions, VEGF release (pg/1000 cells *10^3) was significantly increased by hypoxia (normoxia 37 ± 27 vs. chemical hypoxia: 125 ± 58 and soft hypoxia: 126 ± 41). However, the tubule formation in matrigel (µm) of EPCs submitted to hypoxia not increased (normoxia: 12772 ± 7911 vs. chemical hypoxia: 14036 ± 8705, drastic hypoxia: 9207 ± 5721 and soft hypoxia: 11077 ± 8037). Surprinsingly, compared to normoxia, EPCs proliferation (μm^2) was increased by soft hypoxia (29 ± 12 vs. 44 ± 22) but not by chemical (30 ± 14) and drastic hypoxia (27 ± 19). The phenotype of EPCs submitted to different techniques of hypoxia was analyzed by flow cytometry but none of the markers explored varied significantly after hypoxia.
Conclusion: Our results indicate that the effects of hypoxia on EPCs viability and angiogenic properties dramatically depend on the hypoxia modalities.

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Characterization of the meta-nitrobiphenyline enantiomers as new potential α2C-adrenoceptor subtype agonists

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Introduction: The imidazoline derivative biphenyline displays an interesting selectivity for α2-adrenoceptors (α2-ARs), in comparison to α1-ARs and imidazoline binding sites. Substitution in the second phenyl ring led to a compound termed meta-nitrobiphenyline (mNBP), which according to microphysiometric assays, exhibits agonist activity at the α2C-AR but neither at α2A- nor at α2B-AR subtypes. In the current study, we examined *in vitro* the respective activity of the two enantiomeric forms of mNBP.
Methods: The (R) – (+) -mNBP and(S) – (−) -mNBP enantiomers were individually synthesized and their properties studied on stably transfected CHO cells expressing the α2A-, α2B- or α2C-AR subtype (CHO/α2A, CHO/α2B and CHO/α2C). Binding experiments were conducted using [^{3}H] -RX821002. Microphysiometry, cAMP production and Erk1/2 phosphorylation were respectively determined using a cytosensor (Molecular Devices), RIA and western blotting.
Results: Binding experiments indicated that the rank order of affinity of both enantiomers for α2-AR subtypes was similar: α2A > α2C > α2B. Microphysiometric assays detected agonist activity at the α2C-AR only, for both compounds. In CHO/α2A or CHO/α2B, neither (R) – (+) -mNBP nor(S) – (−) -mNBP were able to modulate forskolin-induced cAMP production. In CHO/α2C,(S) – (−) -mNBP was also ineffective, but (R) – (+) -mNBP inhibited forskolin-induced cAMP production, to an extent similar to that of the α2-agonist dexmedetomidine or of the endogenous agonist, norepinephrine. Examination of Erk1/2 phosphorylation indicated that (R) – (+) -mNBP behaves as a very weak agonist in CHO/α2A and CHO/α2B and as a full agonist in CHO/α2C cells. The(S)- (-) -mNBP had almost no effect on Erk phosphorylation in both CHO/α2A and CHO/α2B, but acts as a partial agonist on Erk signaling pathway in CHO/α2C.
Conclusion: This study indicates that, given their divergent agonist activity towards α2-AR subtypes, the two meta-nitrobiphenyline enantiomers may be in the future interesting tools to delineate the role of α2C-AR *in vivo*. They should be therefore considered as a promising family of compounds, able to selectively target a subtype of α2-AR and/or a specific transduction pathway.

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Hypotensive and anti-aggregant activities of extracts from *Solanum torvum* (Solanaceae) fruits in rat

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Introduction: *Solanum torvum* is a plant commonly used in traditional medicine for the treatment of many diseases including gastric ulcer, intestinal problems, animal bites and arterial hypertension. The present work was undertaken to

evaluate the hypotensive activity of the aqueous and methanol extracts from *S. torvum* fruits. The anti-aggregant activity of the aqueous extract was also evaluated on platelets isolated from rats.
Methods: The hypotensive activity of the aqueous and methanol extracts from *S. torvum* fruits was evaluated in anaesthetised rats using the invasive method. The extracts were administered intravenously at the doses of 1, 2 and 5 mg/kg and the blood pressure and heart rate were monitored for a subsequent 20 minutes. The activity of the aqueous extract was tested in absence and in presence of atropine (1 mg/kg) or yohimbine (1 mg/kg). The anti-aggregant effect of the aqueous extract (0.5, 1 and 2 mg/mL) was essayed on platelet aggregation induced either by thrombin (0.5 U/mL) or adenosine diphosphate (5 μM).
Results: Intravenous administration of the aqueous extract of *S. torvum* induced a dose dependant reduction in arterial blood pressure. The systolic blood pressure reduced by 16.91%, 26.57% and 60.57% at respective doses of 1, 2 and 5 mg/kg. Only the dose of 5 mg/kg significantly affects the heart rate, reducing it by 51.35%. The methanol extract also reduced blood pressure with a percentage fall of 33.47, 59.31 and 49.93 at respective doses of 1, 2 and 5 mg/kg. In contrast to aqueous extract, the methanol extract reduced the heart rate by 21.27%, 20.65% and 29.46% at respective doses of 1, 2 and 5 mg/kg. Neither atropine nor yohimbine significantly affects the hypotensive activity of the aqueous extract. Meanwhile, yohimbine almost completely inhibited the cardiac effect of the extract at the dose of 5 mg/kg. When essayed on the platelet aggregation, induced by either thrombin or Adenosine diphosphate at the concentration of 0.5, 1 and 2 mg/ml, the aqueous extract of *S. torvum* fruits significantly inhibited in dose dependant manner the aggregation induced by both agents, with inhibitory percentages ranging from 29 to 56. This effect did not significantly differ with the aggregant agent.
Conclusion: The methanol extract of *S. torvum* possesses hypotensive activity which may result at least partially from its bradcardiac effect. As the aqueous extract at the doses of 1 and 2 mg/kg reduced blood pressure without affecting the heart rate, it can be thought that its hypotensive effect might not be related to its cardiac action. The anti-aggregant effect of the aqueous extract may be benefit for its cardiovascular effect. The fact that this anti-aggregant activity do not depend to the aggregant agent inferred that the extract may be acting on the step of aggregation common to both agent.

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Quantification and characterization of reactive oxygen species in a model of cocaine-induced cardiac dysfunction using electron paramagnetic resonance spectroscopy

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Introduction: Cocaine use involves many adverse effects on the cardiovascular system by causing myocardial ischemia, arrhytmia, dilated cardiomyopathy and cardiac hypertrophy. Among the involved biochemical mechanisms, the role of myocardial oxidative stress is clearly demonstrated as an early triggering event. However, no direct evidence has been documented so far. In this work, we used electron paramagnetic resonance (EPR) to characterize cocaine-induced myocardial reactive oxygen species (ROS) production, more precisely peroxynitrite and superoxide levels and to investigate the subcellular site(s) involved in this production.
Methods: Wistar rats were treated with saline solution (control) or cocaine hydrochloride (2x7.5 mg/kg/day IP) for seven days. Cardiac function was evaluated by echocardiography. ROS production was measured by EPR spectroscopy in conjunction with the spin probe 1-hydroxy-3-methoxycarbonyl-2,2,5,5-tetramethylpyrrolidine (CMH), with or without mercaptoethylguanidine, a peroxynitrite neutralizer. These evaluations were made in LV homogenates and in two distincts populations of mitochondria: subsarcolemmal mitochondria (SSM) and interfibrillar mitochondria (IFM) in absence and in presence of substrates to initiate respiration.
Results: Cocaine-induced cardiac dysfunction is characterized by a decrease in cardiac index and in LV fractional shortening. In this context, myocardial ROS levels are increased in cocaine rats compared to controls, as evaluate by the rate of CM* formation. Addition of MEG in the assay almost completely abolished the spectra indicating that formation of CM* is in part mediated by the peroxynitrite. In subcellular fractions, substrates-fueled mitochondria generated free radicals is above that in the absence of substrates. In these conditions with substrates supplementation, the EPR signal is markedly increase in IFM from cocaine rats compared to controls while free radicals production from SSM is not different between the groups.
Conclusion: These results demonstrate that cocaine-induced cardiac dysfunction is associated to an increase in myocardial peroxynitrite and superoxide levels. Moreover, this study also suggests a critical role for IFM as an important site of intracellular ROS production.

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Pharmacological postconditioning with the phytoestrogen genistein

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Introduction: Although the cardioprotective effects of hormone replacement therapy remain controversial, acute treatment with estrogens are well known to reduce myocardial infarct size through non genomic effects. Because a cardioprotective strategy is more clinically relevant when instituted at reperfusion, we investigated whether estrogens also induce a pharmacological postconditioning through a PI3K/Akt pathway which is known to protect the myocardium against cell death. The effects of the phytoestrogen genistein and those of 17β-estradiol administered at reperfusion were examined on a rabbit model of coronary artery occlusion (CAO) followed by reperfusion (CAR).
Methods: Pentobarbital-anesthetized rabbits underwent a 20 min CAO followed by 4 h of CAR. Prior to CAR, they randomly received an i.v. Injection of either saline (Control), 100 or 1000 μg/kg of genistein (Geni100 and Geni1000, respectively), and 10 or 100 μg/kg of 17β-estradiol (17β10 and 17β100, respectively). We used low doses of genistein to avoid tyrosine kinases inhibition. Myocardial infarct size (IS) was measured by triphenyltetrazolium chloride staining and expressed as percentage of the area at risk. In additional rabbits sacrificed at 10 min CAR, immunoquantification of phospho-Akt as well as the investigation of the mitochondrial transition pore permeability (mPTP) were performed.
Results: IS was significantly reduced in Gen100 ($n = 6$), Gen1000 ($n = 6$) and 17β100 ($n = 8$) but not in 17β10 ($n = 7$) vs. control ($n = 12$) (6 ± 2, 16 ± 5, 12 ± 3 and 29 ± 7% vs. 36 ± 4%, respectively). Decrease in TUNEL-positive nuclei within infarction, inhibition of calcium-induced opening of mPTP and increase in Phospho-Akt were observed in the Gen100 hearts vs. Control. In addition, the reduction of IS was abolished by the estrogen receptor antagonist fulvestrant (1 mg/kg i.v.) and the PI3K inhibitor wortmaninn (0.6 mg/kg).
Conclusion: Besides the well known genomic effect of estrogens, we demonstrate for the first time that both estrogens and phytoestrogens can induce pharmacological postconditioning by stimulating the estrogen receptors, activating PI3K/Akt and inhibiting mPTP.

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Hypotensive and vasodilator effects of hydroalcoholic extract of *Tapinanthus dodoneifolius* (DC) Danser (Loranthaceae)

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Introduction: Effects of hydroalcoholic extract of *Tapinanthus dodoneifolius* and of its different fractions obtained by partition through column chromatography were investigated on rat blood pressure and aortic relaxation.
Methods: The extract of dried and powerded plants of *Tapinanthus dodoneifolius* in ethanol was centrifuged, lyophilized and fractionated on column chromatography. The crude extract and the fractions obtained were tested on blood pressure in the anaesthetized rat and on relaxation of rat aortic ring precontracted by norepinephrine.
Results: The hydroalcoolic extract of *Tapinanthus dodoneifolius* significantly decreases blood pressure in normotensive rats. Injection of 10 mg/kg of crude extract decreases the control systolic and diastolic blood pressure by respectively 35.5 ± 8.3% and 55.2 ± 12.2% ($n = 4$), but without any significant effect on the heart rate. On aortic smooth muscle contracted by exposure to 1 μM norepinephrine, the crude extract (0.01–3 mg/ml) dose-dependently causes relaxation, with an EC_{50} value of 246 μg/mL and a complete relaxation with 3 mg/ml. After partition of the crude extract by increasing gradient of elution on a column chromatography with dichloromethane/ethyl acetate and ethanol/water solutions, the activity of the 14 obtained fractions was tested on precontracted aortic rings. At the concentration of 1 mg/mL, maximum relaxation is obtained with fractions 2–5; the most active fraction F4 elicits a dose-dependent relaxation (EC_{50} = 160 ± 11 μg/ml, $n = 5$). In addition, the vasodilation is not prevented in the presence of Indomethacin (10^{-5} M) or L-NAME (10^{-4} M) suggesting that this effect is not mediated through cyclooxygenase or NO synthase pathways. Tested on rat blood pressure, 1 mg/kg of fraction F4 decreases systolic and diastolic control values by 58.7 ± 1.7% and 8.03 ± 5.1% respectively, without change on the heart rate.
Conclusion: Our results show that hydroalcoholic extract from *Tapinanthus dodoneifolius* has significant hypotensive and vasodilator effects on rat cardiovascular system. Interestingly, fractionating of the crude extract by chromatography has allowed to separate the active compound(s) in specific fractions. This should lead to their identification, which would be of peculiar interest for development of novel antihypertensive drugs.

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Presence of enterobacteria resistant to quaternary ammonium compounds in faeces of healthy volunteers

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Introduction: Quaternary ammonium compounds are frequently used biocides. Members of the genus *Pseudomonas* are highly resistant leading to the development of Cetrimide agar as a selective medium for the isolation of *Pseudomonas*. During routine use of this medium we stated frequent growth of enterobacteria. The aim of our study was to count and identify enterobacteria resistant to Cetrimide in the faeces of healthy controls in 2003 and 2005.
Methods: In 2003, 49 healthy volunteers were sampled and 67 in 2005. Fresh voided faeces were analyzed within 4 h and plated on Cetrimide agar and on McConkey agar, medium selective for enterobacteria. Species were identified using the API 20E system.
Results: In 2003, 20% of samples showed no growth on Cetrimide agar compared to 2% in 2005 ($P < 0.01$). The mean number of species was 1.1/sample in 2003 rising to 1.5/sample in 2005 (NS). The mean count was 4.04 ± 0.71 log CFU/g of faeces in 2003, reaching a level of 4.75 ± 0.95 log CFU/g in 2005 ($P < 0.01$). The most frequent species *Escherichia coli* was isolated in 53% of samples in 2003 but 79% in 2005 ($P < 0.01$). When comparing counts on McConkey and Cetrimide agar, we stated that only a small proportion of *E. coli* are resistant to Cetrimide, while the other genera isolated (*Citrobacter, Hafnia, Enterobacter, Klebsiella*) are present at equal levels on the two media.
Conclusion: Increasing levels of enterobacteria resistant to quaternary ammonium compounds are found in the faeces of healthy volunteers. Can the frequent use of the compounds in disinfectants and cosmetics induce resistance in enterobacteria? Further studies are necessary to explain the mechanism of resistance. Quaternary ammonium compounds seem no longer efficient as disinfectants and the evolution of these resistances should be followed.

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Quantification of pharmacodependence seriousness: status of validation of a novel tool developed by Nantes CEIP

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Introduction: Pharmacodependence notifications issued by professionals are collected by each CEIP which evaluates them: in order to harmonize case reporting and make it possible to homogeneously measure the seriousness of pharmacodependence cases, Nantes Center for Evaluation and Information on Pharmacodependence (CEIP) has developed a novel method which, for the first time, quantifies,

out of notification data, patient pharmacodependence behaviour by calculating a seriousness score for any drug or substance. The proposed poster describes the current status of the validation process which consist in benchmarking pharmacodependence seriousness of targeted drugs, against substances whose dependence potential is well documented.
Methods: The method consists of a questionnaire which includes (i) a series of questions derived from the items of DSM-IV of the definition of pharmacodependence, and (ii) an additional set of specific questions (created by Nantes CEIP), aimed at evaluating patient transgression behaviours (fraud and misuse). The answers are then processed to calculate a score of pharmacodependence seriousness for each substance targeted in the notifications. Criteria including the content validity and the construct validity have been integrated.
As part as the validation process of the method we processed notification data related to targeted drug and compared it to data related to known substances.
Results: During the validation process, the method has already produced several scores which allow an objective measurement of pharmacodependence seriousness of drugs, regardless of their previously known dependence potential: for instance we found that some drugs show a higher seriousness score than some illicit substances such as heroin.
Conclusion: The association of clinical data generated by the tool, and official data coming from CEIP's, represents a such powerful combination that all French CEIP's contribute to the approval process of the method. As French CEIP receive all together about 1800 notifications each year, Nantes CEIP will soon have a very valuable material to validate its novel tool.

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Re-evaluation of drug treatment in the elderly: it it necessary and possible ?
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Introduction: Polypathology, polypharmacy and increased risk for adverse events are the main factors which encourage to regularly re-evaluate drugs prescriptions in the elderly. The aim of this study was to do a quantitative and qualitative analysis of the drugs modifications for chronic diseases in hospitalized patients aged more than 70 years.
Methods: Four hundred and ninety-four patients (83.4 ± 7.4 years, women 64.6%) consecutively admitted during 6 months in 2005 were included. We did not included patients admitted for palliative care and patients died during the hospitalization. We recorded all the drugs administered before admission and at discharge. We did consider neither the drugs administered for an acute disease (infection) or dosage modifications. Each drug was classified in one of 14 drugs groups according to its general indication (cardio-vascular system, neuro-psychiatry, metabolism-nutrition, pain…). Then we compared all the drugs between admission and discharge. For each modification, we recorded one reason or more for modification: disease not previously treated, no indication or disappeared indication, adverse event, high risk for adverse event or dangerous drug–drug interaction, therapeutic optimization, bad compliance, not proved efficacy, better presentation, physician's habit, too expensive drug, placebo effect, not available drug.
Results: The 494 patients received 2908 drugs at admission (5.8 per patient) and 2832 at discharge (5.7 per patient), without significant difference, but 36.5% of drugs were actually modified between admission and discharge. The main prescribed drugs were 'cardio-vascular' (42% at admission), 'neuro-psychiatric' (19%), 'metabolism-nutrition' (13.9%) and 'gastro-enterologic' (10.7%) drugs. The percentages of modified drugs were 32.3% of 'cardio-vascular' drugs, 40.1% of 'neuro-psychiatric' drugs, 33.7% of 'metabolism-nutrition' drugs, 41.7% of 'gastro-enterologic' drugs, 68.4% of analgesics, 22% of 'pulmonary' drugs and 30.3% of 'rheumatologic' drugs. 1774 reasons for modifications were recorded. The main reasons for drugs modifications were: disease not previously treated (32.2%), no indication or disappeared indication (26.6%), therapeutic optimization (17.1%), adverse event (6.3%), not proved efficacy (4.8%), high risk for adverse event (3.3%). But the frequencies of reasons for modifications differed between the different drug classes.
Conclusion: The re-evaluation of drug administered for chronic disease in the elderly is very important because more than one third of drugs were modified during the hospitalization. Diseases not previously treated and absence or disappearance of indication were the more frequent reasons for drugs modifications. This result explain why mean drug number did not differ between admission and discharge.

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Quality of life questionnaires in skin diseases: use in randomized clinical trials
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Introduction: The European Regulatory Issues on Quality-of-Life Assessment (ERIQA) working group published recommendations on the use of QOL questionnaires as an evaluation endpoint in randomized clinical trials (RCTs). The objective of this systematic review was to gather the reports on RCTs measuring QOL in dermatology, and analyze them based on the ERIQA recommendations.
Methods: A computerized bibliographic search limited to RCT, with the key words « quality of life and skin diseases » was realized on Medline, Cochrane Library et Embase from 1966 to February 2005. The studies using at least 1 QOL questionnaire, concerning skin diseases and written in English or French were selected. We excluded studies concerning cancers, and human immunodeficiency virus (HIV). We analyzed each retained RCT according to the ERIQA checklist.
Results: A total of 910 publications were extracted from (Medline (n = 392), Embase (n = 200), and Cochrane Library (n = 318) 757 reports were exluded based on their abstracts, because of search overlap (n = 303), other language (n = 2), review (n = 3), no dermatological diseases, dealt with HIV or cancer (n = 449). On the 153 remaining reports, 110 were excluded after fully review: no RCT (n = 13), QOL evaluation (n = 32), no QOL questionnaire (n = 65). The remaining 43 articles were analyzed. The first analyzed study was published in 1993, 77% (33) of the analyzed studies were published between 2001 et 2004. Twenty distinct QOL questionnaires were used. The Dermatology Life Quality Index (DLQI) used in 37% (16) was the most frequently used questionnaire. 72% (31) of the 43 RCT were double-blind. QOL was the aprimary judgment criterion in six studies. 12% (5) of the retained trials justified the choice of a general or specific questionnaire. Ninteen percentage (8) of the reports described the questionnaire. Seven percentages (3) of the articles included a hypothesis of a QOL-score change with calculation of the number of subjects needed. Statistical analysis was performed on an intent-to-treat basis for QOL in 33% (14) of the RCTs. 26% (11) of the reports addressed the problem of missing data. Results were presented as means ± standard deviation of the overall scores and for each dimension for each group studied as recommended in 9% (4) of the papers. No statistical differences between treatments for QOL was found in 42% (18) RCTs.
Conclusion: Our analysis highlighted many methodological weaknesses in the use of QOL questionnaires in RCTs.

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Local survey of long-acting injectable risperidone prescription practices
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Introduction: The 31 january 2006 report of the French National Commission of PharmacoVigilance related 39 observations, since march 2004, of delirium or hallucination increase and therapeutic failure with long-acting injectable risperidone. These adverse effects could be linked to a drug misuse and has led to modify the drug monograph. Then, we have decided to lead a survey about long-acting risperidone prescription practices in our hospital.
Methods: Our retrospective survey was possible because of the nominative traçability of long-acting injectable risperidone dispensation at the hospital pharmacy. Thus, all the prescriptions of long-acting risperidone initiated in our hospital have been gotten out from july 2005 (when the drug was available in our hospital) to august 2006. We have elaborated an appraisal form. And for each patient, all the data required have been collected from prescriptions and analysis of clinical file on the one hand and from interviews of psychiatrists and nurses on the other hand.
Results: A total of 31 patients have been included and followed. With regard to prescription initiation practices, three mains criteria have been checked: effective evaluation of the required dose of oral risperidone before long-acting drug setting up (61.3% in accordance with recommendations), coherence of doses between oral and injectable switch (64.5%), respect time of overlap (70%). But the all three criteria was together in line with recommandations in only 35.5% of cases. With regard to prescriptions evolution practices with long-acting risperidone treatment, interesting results appear. After a while and whatever the initial long-acting risperidone dose, 93.5% of patients received the maximal dose of 50 mg/2 weeks. Whatever the oral risperidone dose required at the beginning, the long-acting risperidone treatment has been continued in effective antipsychotic monotherapy for 32.3% of patients. Regarding patients who received an oral risperidone dose at 6 mg or less, 42.1% of patients have continued their treatment in effective antipsychotic monotherapy. Whereas, they have been only 16.7% when the initial oral risperidone dose was higher than 6 mg.
Conclusion: Thus, the results analysis confirms the drug monograph modifications requested by the National Commission of PharmacoVigilance report: a treatment with long-acting injectable risperidone does not appear interesting in patients stabilized by oral risperidone dose higher than 6 mg. Moreover, our results show that we have to increase information regarding treatment initiation modalities. In that way, a detailed information was brought to the psychiatrists during the last hospital drug committee.

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The Paediatric CIC Network: Year 2006
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Introduction: The Network of Paediatric Clinical Investigation Centres is a national network set up in 2001, consisting of seven centres. The network aims at stimulating clinical research, both investigator-initiated and industrially sponsored, in the area of drug evaluation in children and at participating in training and education.
Methods: They are integrated into teaching hospitals and collaborate with medical and technical departments, Inserm and university research units. The CIC.P contributes to technical innovations, has facilities designed for the conduct of research in children and provides support to investigators, from design through the conduct of clinical research protocols. The CIC network also supports parents and their children during participation in medical research.
Results: One hundred and twelve protocols, either single center, multicentre at the national level or with international sites, were ongoing in year 2006 in all paediatric subspecialties including neonatology, endocrinology, hepatology-oncology, paediatric surgery.
Conclusion: The CIC.P network already provides pharmaceutical industries with competences in paediatric research and facilities specially designed for the conduct of clinical research in children that will facilitate the answer to the new European Paediatric Regulation aiming to increase the development and authorisation of medicinal products for use in children to ensure that children's medicinal products are subject to high quality research and children are not subjected to unnecessary clinical trials.

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Beneficial hemodynamic effects of activated protein C in a rodent model of endotoxic shock
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Introduction: Cardiovascular dysfunction is one of the determinants of mortality in the context of septic shock. Our question was: does activated protein C (APC) has hemodynamic effects in a rodent model of septic shock?
Material and methods: Male Sprague–Dawley rats (250–275 g). Internal jugular vein and carotid artery were tunnelled subcutaneously for arterial pressure measurment and drugs administration. Four groups were studied:
Controls: physiologic saline at a rate of 2 ml/h

Controls + APC: 240 μg/kg/h diluted in saline at 2 ml/h rate
LPS: physiologic saline at a rate of 2 ml/h and 10 mg/kg of endotoxin of *E.coli*
LPS + APC: 240 μg/kg/h diluted in saline at 2 ml/h rate started 30 minutes before LPS administration at 10 mg/kg
We measured arterial pressure during 4 hours. Serum samples were taken for determination of TNFalpha levels, nitrate/nitrite levels and MIF (macrophage inhibiting factor). Left ventricular pressure and it's first derivative dP/dtmax was assessed by a latex balloon inserted in the left ventricle connected to a pressure transducer. Statistical analysis was analysis of variance. When a significant difference was found, we examined between-group differences using a sequentially rejective Bonferroni procedure. The level of statistical significance was set at $P < 0.05$ for comparisions with controls.
Results: In the LPS group there is a drop in mean arterial pressure over the time during the 4 hours. APC reduced LPS-induced drops in arterial pressure over time 107 ± 7 mmHg vs. 82 ± 6 mmHg ($P < 0.05$).
APC prevented LPS induction of contractility decrease at H4LVEDP 82 ± 4 mmHg for APC treated and 60 ± 3 mmHg for LPS ($P < 0.05$). dP/dtmax 3300 ± 90 mmHg/s for APC treated and 2700 ± 80 for LPS ($P < 0.05$). The dose-response of arterial pressure increase function of dose of norepinephrine was flat for the LPS group. A complete recover was seen in APC treated animals. 55 ± 6% de delta of artérial pressure for APC treated rats compared with 10 ± 7% for LPS rats and 37 ± 8% for controls ($P < 0.05$). TNFalpha is less increased in APC group (130 ± 23 ng/mL vs. 230 ± 30), plasmanitrates/nitrites too (6.56 ± 2.10 vs. 14.79 ± 1.54), and MIF too (60 ± 8 ng/mL vs. 115 ± 23).
Conclusion: In a rodent model of septic shock, activated protein C prevents cardiovascular dysfunction (contractility, mean arterial pressure), vascular reactivity, and reduced increasing of plasmatic levels of TNFalpha, nitrates/nitrites, MIF.

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Cell surface expression of LDL Receptor is closely related to viral load in HCV-infected patients
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Introduction: The control of hepatocyte viral penetration may be an important key in hepatitis C treatment. The LDL receptor (LDL-R) has been proposed as the viral receptor for Hepatitis C virus (HCV).This hypothesis has been based exclusively on *in vitro* studies. In human mononuclear cells, LDL receptor gene expression has been demonstrated to parallel and be coordinately regulated to gene expression in human liver. The purpose of the current study was to determine the mononuclear cell surface expression of LDL receptor in patients with HCV chronic infection according to viral load.
Methods: Sixty-eight consecutive untreated chronic hepatitis C patients were studied to determine the mononuclear cell surface expression of LDL Receptor. LDL-receptors were quantified at the surface of mononuclear cells in fresh fasting blood samples by a flow cytometry method.
Results: LDL-receptor expression was significantly associated with LDL-cholesterol *($R = -0.25$; $P = 0.03$)* and HCV-viral load *($R = 0.37$, $P = 0.002$)*. In multivariate analysis, HCV viral load was significantly associated with LDL-receptor expression, whereas genotype, age, BMI and fibrosis were not.
Conclusion: Our data strongly suggest that one of the crucial parameter for viral entry would therefore be the LDL receptor at the cell surface of the hepatocytes. This result may open a new therapeutic research field in chronic hepatitis C.

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Effectiveness of lamivudine in a cohort of HIV and hepatitis B co-infected patients
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Introduction: Data on the effectiveness of drug therapies aimed to slow the progression of hepatic disease is sparse in patients co-infected with human immunodeficiency virus (HIV) and hepatitis B virus (HBV).
Methods: We prospectively followed in our hospital a cohort of 107 HIV-HBV co-infected patients of various geographical origins and ways of transmission, identified among attending HIV patients, to study the relation between administered antiviral treatments and hepatic outcome. Clinical examination, liver and virological tests were performed every 3–6 months. 44% of patients had a liver biopsy. Mean follow-up was 5.3 years.
Results: Two geographic origins (33% Europeans, 61% Sub-Saharan Africans) and two HBV genotypes (48% genotype A, 43% genotype E) predominated. During follow-up, 11 patients died, of whom four from HBV-related liver disease (4%, 0.7/100 patient-years). Advanced liver disease developed in 19 patients (18%, 3.3 cases per 100 patient-years): 3 hepatocarcinoma, five cirrhosis, 10 extensive fibrosis (Metavir F 3 or 4), and one fulminant hepatitis after stopping lamivudine.
Twenty-one percentages of patients received no antiviral therapy during follow-up. Of treated patients, 96% (78% of the cohort) received lamivudine at some moment. Lamivudine was combined with or followed by adefovir or tenofovir in 26% of patients.
Twenty-eight percentages of patients not receiving lamivudine, or receiving it for <1 year, developed advanced liver disease, compared with only 12% of patients treated with lamivudine for more than 1 year (relative risk 2.3, 95%CI 1.01–5, $P = 0.04$). Of the 19 patients who developed advanced hepatic disease, 12 (63%) had received lamivudine before the diagnosis, for a mean of 15.5 months (SD 19). Cumulated time receiving lamivudine was one of the two main predictors of developing hepatic advanced disease identified in a multivariable Cox model, with a protective effect (Hazard Ratio (HR) per month of treatment, 0.96; $P < 0.001$). The other main predictor was elevated mean transaminases (HR per 10 UI/mL increase, 1.88; $P < 0.001$).
Lamivudine was discontinued in 31 patients, 7 (23%) presented an hepatitis flare shortly after, which in 1 case evolved into a lethal hepatitis. The risk of developing resistance mutations correlated well with the total time receiving lamivudine (Odds Ratio per month of treatment 1.03, 95%CI 1.01–1.06, $P = 0.005$).
Conclusion: Lamivudine remains effective in HIV–HBV co-infected patients, but some subgroups of patients (i.e. With persistently elevated transaminases) have a high frequency of advanced liver disease at 5 years of follow-up despite this treatment. Better therapeutic strategies are needed for those high-risk groups.

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Rifamycin lavage in the treatment of experimental intra-abdominal infection
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Introduction: To investigate the effect of peritoneal lavage with rifamycin to reduce the number of intra peritoneal bacteria and adhesion and to improve the outcome of intra abdominal infection (IAI).
Methods: Experimental IAI was induced in Wistar rats using the cecal ligation and puncture model. After 24 hours, the animals underwent relaparotomy with cecal excision. Peritoneal fluid sample was obtained and lavage of the abdominal cavity was performed. Animals were randomly assigned to three groups. S group: lavage with 0.9% sodium chloride solution, R25 group: lavage with rifamycin at the dose of 25 mg/kg and R12.5 group: lavage with rifamycin at the dose of 12.5 mg/kg. Mortality was recorded every 8 hours for 7 days. All animals that died had a necropsy. Surviving rats were later sacrificed and also underwent a necropsy. At necropsy, intraperitoneal adhesions were noted and peritoneal fluid sample was obtained. Bacterial and leucocytes counts from peritoneal fluid were measured.
Results: Peritoneal lavage with rifamycin reduce mortality from 50% in the S group to 87.5% and 100% in the R25 group and R12.5 group respectively. Adhesion formation was significantly reduced in the R25 group and R12.5 group compared with the S group ($P = 0.01$and $P < 0.01$ respectively).The intra peritoneal adhesion were not significantly different between R25 group and R12.5 group. There was a greater reduction in bacterial counts in peritoneal fluid in the R25 group compared with the S group ($P = 0.037$) but there was no significant difference in the reduction of bacterial count between R25 group and R12.5 group and between S group and R12.5 group.
Conclusion: These results suggest that peritoneal lavage with rifamycin reduce mortality, the number of adhesion and the bacterial counts and might be useful in the treatment of IAI.

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***In vitro* effect on TNF-alpha and IL-10 production by activated peripheral blood mononuclear cells from Crohn's disease patients by different lipid emulsions for parenteral use with a various n-6/n-3 polyunsaturated fatty acids ratio**
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Introduction: Fish oil-derived n-3 PUFAs have prompted a growing interest in human therapeutics as potential inflammatory and immune regulators. Nevertheless, until now, when administered orally, their benefit in inflammatory diseases treatment seems not to be of major impact.
Therefore, we studied the *in vitro* modulation of lipopolysaccharide (LPS) -activated PBMCs TNF-alpha and IL-10 production by a 100% n-3 containing lipid emulsion (Omegaven®, Fresenius Kabi) and a soybean oil-derived lipid emulsion with a 7.4 n-6/n-3 PUFAs ratio (Endolipide®, Baxter Clintec).
Methods: PBMCs have been obtained from peripheral blood from fasting Crohn's disease (CD) patients and healthy controls (HC). PBMCs (1 million/mL/well; activated by 10 μg/mL of LPS from *Salmonella abortus* equi) were cultured at 37°C [humidified air (95%) / CO_2 (5%) atmosphere] in RPMI 1640 containing 10% fetal bovine serum, 2% L-glutamine and 1% antibiotics (streptomycin/penicillin), in the presence or not of 0.01%, 0.1% and 1% of either Omegaven® or Endolipide®. After 24-hours of culture, supernatants were removed and stored at -80°C until cytokine measurement by ELISA (R&D Systems).
Results: (1) Endolipide® did not influence TNF-alpha production by LPS-activated PBMCs from HC and CD patients; noteworthy, Omegaven® at 1% slightly decreased *in vitro* TNF-alpha concentrations in culture supernatants. (2) Both Omegaven® and Endolipide® inhibit strongly and dose-dependently IL-10 production by HC and CD patients PBMCs. (3) Finally, previous studies using HC PBMCs suggest that a more balanced n-6/n-3 ratio [i.e. 60% Endolipide® – 40% Omegaven® (n-6/n-3: ~0.9)] may be more efficient in *in vitro* TNF-alpha inhibition (~78% inhibition compared to LPS-activated PBMCs TNF-alpha production in the absence of any PUFAs).
Conclusion: By contrast to lipid emulsions for parenteral use with a high n-6/n-3 PUFAs ratio which appear not to be able to modulate LPS-activated PBMCs cytokines production [1], lipid preparations for IV use with a more balanced n-6/n-3 ratio may inhibit *in vitro* TNF-alpha production from both HC and CD patients LPS-activated PBMCs. Both n-6/n-3 ratios (n-6/n-3: 7.4 or 100% n-3) significantly inhibit IL-10 production in the same experimental conditions.
These *in vitro* data, taken together with recent beneficial results reported by administering Omegaven® intravenously in active rheumatoid arthritis patients [2], need to be considered and to be confirmed before potential (nutritional or therapeutic) use of IV n-3 PUFAs in CD patients.

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Comparison of France vs. the rest of the World on use of venous thromboembolism prophylaxis in patients hospitalized for acute medical illness: a prospective register
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Introduction: Although it has been showed that tromboprophylaxis reduced mortality in patients hospitalized for acute medical illness, an observational cohort with multinational sites is of importance to assess the thromboprophylaxis use in clinical practices.
Methods: Eligibility criteria: Patients >18-years old, hospitalized at least 3 days for an acute medical illness, and not receiving an antithrombotic therapy at curative dose.

Data collection: Enrollment form during the hospital stay; follow-up for venous thromboembolic (VTE) events occurring within 3 months after hospital discharge.
Results: Data result from 52 active sites in 12 countries. Recruitment began in July 2002 and ended in Sept. 2006.
Enrollment cases: 1605 (France); 14871 (World)

	France	World		France	World
Female	52%	50%	Prophylaxis used	60%	51%
Age (median)	73	68	LMWH (**)	47%	34%
Heart disease	15%	16%	Unfractioned heparin	7%	11%
Lung disease	16%	23%	In-hospital VTE	1%	1%
Neuro disease	28%	12%	3 months VTE	1%	1%
Low-dose aspirin	21%	26%	In-hospital bleeding	5%	8%
NSAIDs (*)	2%	16%	3 months bleeding	2%	3%

(*) Non steroidal anti-inflammatory drugs (including regular-dose aspirin)
(**) Low molecular weight heparin.

Conclusion: Medical in-patients in French hospital received more VTE prophylaxis with LMWH, compared to the rest of the world.
This prophylactic strategy has no clear effect on VTE incidence and on bleeding events.

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Ex vivo biotransformation of carbamazepine and one of its metabolite iminostilbene in physiological conditions

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Introduction: Carbamazepine (CBZ) belongs to the group of major anticonvulsive agents but is associated with serious adverse reactions. The metabolism of carbamazepine involves several enzymatic pathways. One of them implicates iminostilbene (IM), the decarboxamide derivative of CBZ. The mechanism of adverse reactions is poorly known but includes probably white cells toxicity and/or activation, through the formation of reactive metabolites. *In vitro* biotransformation of CBZ by activated neutrophils has been demonstrated (1). In the same study, the authors showed that neutrophils were able to convert iminostilbene (IM) to reactive intermediates as 9-acridinecarboxaldehyde, acridine (AI) and acridone (AO). Therefore *in vitro* conditions used in this study were drastically oxidative using hydrogen peroxide, chloride and exogenous myeloperoxydase. Thus, in our study we performed experiments in more physiological conditions in order to evaluate *ex vivo* biotransformation of CBZ and IM.
Methods: Blood samples (n = 30) were obtained from carbamazepine treated patients. Each sample was divided into 12 fractions of 500 µL to test influence of drug addition on the formation of AO. CBZ (33 µM and 100 µM) and IM (10 µM and 33 µM) were the tested drugs (final concentration). Incubation of blood samples at 37°C in oxygenated conditions was performed during 6 hours. Plasma was then aliquoted after centrifugation and frozen until dosage. To evaluate the spontaneous degradation of CBZ and IM, separates aqueous solutions (200 µM) of each drug were tested for stability for 4 days (control samples). LC-MS quantification was used to determine CBZ, IM and AO concentrations at three times (30 minutes after the beginning of the incubation, 3 hours later and at the end of incubation).
Results: No increase of AO concentration was observed with CBZ stimulation in any condition. In contrast, AO concentrations increased with croissant addition of IM in blood samples. IM concentrations decreased with incubation time but not AO. No AO formation occurred in the control sample and IM concentration was stable. These results are in accord with a very rapid formation of AO from IM. The decrease of the IM concentration could be explained by the strong affinity of IM for the lipidic fraction of the sample which is removed by extraction procedure. Further experiments are needed with other metabolites of CBZ as epoxycarbamazepine and AI.
Conclusion: These data strongly suggest a biotransformation of IM into AO in the blood of epileptic patients in physiological conditions.

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The influence of treatment and anamnestic factors recorded at diagnosis on the outcome of patients with hepatitis C: a population based-study

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Introduction: Despite numerous descriptions, the outcome of patients with chronic hepatitis C virus (HCV) infection remains unclear in the general population. The goal of this study was to assess factors recorded at first referral and antiviral therapy as determinants of outcome.
Methods: Population-based study involving two French administrative areas.
Participants: 1161 HCV-positive viremic patients.
1161 HCV-positive viremic patients.
Measurements: factors associated with cirrhosis at baseline, subsequent decompensated cirrhosis, hepatocellular carcinoma, and death (liver-related or not) were determined though multivariate analyses.
Factors associated with cirrhosis at baseline, subsequent decompensated cirrhosis, hepatocellular carcinoma, and death (liver-related or not) were determined though multivariate analyses.
Results: A total of 155 patients had cirrhosis at baseline. At the end of 55 months median follow-up were recorded 127 cases of decompensated cirrhosis, 67 of hepatocellular carcinoma, and 170 deaths (62 liver-related). The 5-year rates of cirrhosis decompensation, hepatocellular carcinoma, and death liver-related or not were 6.2%, 3.2%, 5.9%, and 6.9%, respectively.
Among variables recorded at baseline, age > 60 years had pejorative influence (OR = 13.8 for baseline cirrhosis; RR = 19.1 for hepatocellular carcinoma; RR = 31.6 for liver-related death and RR = 23.7 for non liver-related death, $P < 0.0001$), together with alcohol consumption (OR = 4.9 for baseline cirrhosis; RR = 4.82 for hepatocellular carcinoma; RR = 3.9 for liver-related death; RR = 1.9, for non liver-related death, $P < 0.0001$), male gender (OR = 2.0 for baseline cirrhosis, P = 0.004), rural place of residence (OR = 1.7 for baseline cirrhosis, P = 0.039; RR = 1.27 for liver related death, P = 0.017), absence of antiviral therapy (RR = 4.6 for liver-related death, P = 0.012; RR = 2.1 for non liver-related death, P = 0.031). Conversely, HCV systematic screening was associated with better outcome (OR = 0.71 for baseline cirrhosis, P = 0.0003; RR = 0.33 for liver related death, P = 0.01).
Conclusion: Old age at HCV diagnosis, alcohol consumption, source of HCV diagnosis different from systematic screening, rural place of residence and absence of treatment were significant determinants of pejorative outcome.

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Evaluation of two evidence-based knowledge transfer interventions for physicians. A cluster randomized controlled factorial design trial: the CardioDAS study

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Introduction: To investigate the potential benefits of two modes of evidence based knowledge transfer ('active' and 'passive' modes) in terms of improvement of intention of prescription, knowledge, and real prescription in practice, we performed an open randomized controlled trial (CardioDAS) using a factorial design (two tested interventions: 'active' and 'passive' knowledge transfer) and a hierarchical structure (cluster of physicians for each department level).
Methods: The participants were cardiologists working in French public hospitals. In the 'passive' transfer group, cardiologists received evidence based knowledge material (available on Internet) every week during 1 year. In the 'active' transfer group, two knowledge brokers (EA, PN) visited the participating departments (every 2 months during 1 year, 2 hours per visit). The primary outcome consisted in the adjusted absolute mean variation of score (difference between post and pre study session) of answers to simulated cases assessing the intent to prescribe. Secondary outcomes were the variation of answers to MCQ assessing knowledge and of the conformity of real prescriptions to evidence based reference assessing the behavioral change.
Results: Twenty-two French units (departments) of cardiology were randomized (72 participating cardiologists). In the 'active' transfer group, the primary outcome was more improved than in the control (P = 0.031 at the department level, absolute mean improvement of five points/100). The change in knowledge transfer (MCQ) was also significant (P = 0.039 at the department level, absolute mean improvement of six points/100). However, no benefit was shown in terms of prescription conformity to evidence. For the 'passive' mode of knowledge transfer and for the three considered outcomes, no improvement was identified.
Conclusion: CardioDAS findings confirm that 'active' knowledge transfer can change evidence from clinical trial capture by doctors. 'Passive' seems far less efficient. However, in this study the changes in acquired knowledge were modest and did not lead to statistically significant improvement in prescription behaviors.

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Proton pump inhibitors vs. histamine 2 receptor antagonists for stress related mucosal bleeding prophylaxis in critically ill patients: a meta-analysis

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Introduction: Stress-related mucosal bleeding (SRMB) causes significant morbidity and mortality. H_2-receptor receptor antagonists (H2RA) have been shown to reduce SRMB rates, yet randomized trials (RCTs) assessing proton pump inhibitors (PPIs) have yielded conflicting results.
Objective: To evaluate the efficacy of PPIs vs. H2RAs in the prophylaxis of SRMB in critically ill adults with risk factors for bleeding.
Methods: Searches of the past four decades in MEDLINE, EMBASE, CENTRAL (Q4-2006), and ISI WEB OF KNOWLEDGE were conducted. Only fully published RCTs published in English were included, if the required data could be extracted. We reviewed all RCTs comparing the efficacy of PPIs to controls (H2RAs, sucralfate, or placebo). Outcomes measured were the decreases in rates of clinically significant bleeding (B, primary outcome of the meta-analysis), nosocomial pneumonia (P), and mortality (M) (secondary outcomes). Study heterogeneity was sought and quantified. Results are reported as odd-ratios (OR) with 95% confidence intervals using a random effect model (Review Manager 4.1).
Results: Three RCTs met the inclusion criteria; of the eight arms contained in these studies, the meta-analysis assessed the six treatments arms that compared PPIs to H2RAs [omeprazole (n = 3), cimetidine (n = 1), ranitidine (n = 1), and famotidine (n = 1)]. Prophylactic PPI administration did not yield any significant decrease in the incidence of bleeding [n = 569 patients, OR 0.41, (0.15; 1.14)]; with no observed heterogeneity (P = 0.26, I^2 = 26.4%). Moreover, no statistical differences were apparent for the development of nosocomial pneumoniae [n = 3, n = 569 pts, OR = 0.72, (0.25; 2.10)], in which moderate heterogeneity was found P = 0.05 I^2 = 66.7%, or for mortality [n = 2, N = 502 pts, OR = 1.35, (0.82; 2.22)], for which there was no observed heterogeneity (P = 0.95, I^2 = 0%). Interestingly, although all studies showed lower rates of bleeding for PPIs vs. H2RAs, only one study with a high rate of bleeding in both groups compared to current general estimates significantly favored their use.
Conclusion: In critically ill patients with recognized risk factors for the development of SRMB, PPI prophylaxis did not significantly decrease rates of clinically significant bleeding, nosocomial pneumonia, or mortality. It is possible that the meta-analysis is currently underpowered to show a significant improvement in bleeding attributable to PPI's. Additional studies are required to definitively exclude any possible benefit, and as initial step, an attempt at getting more complete information from trials published in English only as abstracts is warranted.

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Effect of homeopathy on analgesic intake following knee's ligament reconstruction

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Introduction: The efficacy of homeopathy is still debated. Recent meta analysis recommended to perform further randomised double blind clinical trials to try to identify any clinical situation in which homeopathy would be effective.

The objective of the study was to assess the efficacy of an homeopathic treatment on cumulated morphine intake delivered by PCA (Patient controlled analgesia) 24 hours after a knee's ligament reconstruction.
Methods: We performed an add-on randomized controlled study with three parallel groups: a double-blind homeopathic or placebo arm and an open-label non-interventional control arm.
Eligible patients were 18 to 60 years-old candidates to a surgery of the anterior cruciate ligament by Kenneth-Jones (KJ) or doubled semitendinosus and gracilis tendons (DGST) technique.
Treatment (Arnica Montana, 5 CH, Bryonia alba 5 CH, Hypericum perforatum 5 CH, Ruta graveolens 3 DH) was administered the evening before surgery for 3 days. Primary end-point was cumulated morphine intake delivered by PCA during the first 24 hours inferior or superior to 10 mg/day.
Results: One hundred and fifty-eight patients were randomized (66 in placebo arm, 67 in homeopathic arm and 25 in non-interventional group). The three groups were comparable. There was no difference between the treated and the placebo group for the morphine intake 24 hours after surgery (48% and 56% had <10 mg/day of morphine in each group respectively). The homeopathy had neither an effect on morphine intake between 24 and 72 hours nor on the visual analogic pain scale, nor on the quality of life assessed by the SF-36. In addition, these parameters were non significantly different in patients enrolled in the open-label non-interventional control arm.
Conclusion: Homeopathy is not superior to placebo for post-surgical pain intake after knee ligament reconstruction and shows no placebo effect.

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Effect of general practitioner education on antibiotic prescription: a large scale randomized study
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Introduction: Several studies have shown a strong relationship between antibiotic consumption and frequency of bacterial resistance. Respiratory tract infections are the most common indication for antibiotic prescriptions in primary care. However, the effectiveness of general practitioners education to reduce antibiotic prescribing remains discussed.
Methods: The aim of this large scale randomized trial was to evaluate the impact of general practitioners education on antibiotic prescribing. One hundred and seventy physicians were included in this study. General practitioners randomized in the control group received no specific recommendation on antibiotic prescription. Physicians randomized in the education group attended to a 2 days (16 h) seminar focussed on evidence-based guidelines on antibiotic prescribing in upper and lower respiratory tract infections (September 2004). Data on general practitioners prescriptions were obtained from the database of the French National Health System. We defined the period from January 2004 to March 2004 as baseline and the same data were collected 18 months after the intervention (January 2006 to March 2006).
Results: At baseline, the antibiotic prescription rate was similar between both groups. From 2004 to 2006, we observed a larger decrease in the percentage of prescriptions including an antibiotic in the education group than in controls (relative decrease of −24.7% vs. −11.6% in education and control groups respectively, $P < 0.001$ between both groups). Similarly, the global cost of antibiotic prescribing was significantly reduced in the intervention group (−17.1% vs. −3.7%, $P < 0.05$).
A subgroup analysis showed that the reduction in antibiotic prescribing observed in the education group was significant for both adults (−25.0% vs. −9.6% in education and control groups respectively, $P < 0.01$) and elderly patients (−9.4% vs. +15.4%, $P < 0.01$).
Interestingly, we found opposite results for symptomatic drug prescribing. In 2006, we observed an increase in the prescription of these drugs that was larger in the education group than in controls (+18.6% vs. +11.5% respectively, $P < 0.001$). However, the combined cost of both antibiotic and symptomatic drugs prescribing remained lower in the intervention group ($P < 0.05$).
Conclusion: This randomized controlled trial shows that general practitioners education reduces the prescription of antibiotics in general practice. Our approach could be used as a model for future educational programs aiming to decrease antibiotic use and bacterial resistance.

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Comparison of pramipexole vs. ropinirole on efficacy and tolerability in the restless legs syndrome (RLS): Bayesian indirect meta-analysis
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Introduction:
Objectives: To perform a direct and indirect meta-analysis of the efficacy and tolerability of pramipexole (PPX) and ropinirole (RPR) in the absence of comparative trials. Both treatments are widely approved in RLS.
Methods: Clinical trials were identified from a systematic search and clinical reports. Study inclusion criteria: studies in idiopathic RLS, randomized, double-blind, placebo-controlled, parallel group, primary endpoint: International restless legs rating scale (IRLS). Pre-specified analyses were: fixed and random-effects models for direct comparisons and a Bayesian approach for the indirect comparison, PPX vs. RPR, using placebo as the common comparator. Non-inferiority of PPX vs. RPR was tested first and then superiority. Efficacy criteria were: IRLS mean change from baseline and percentage of responders on the clinical global impressions–improvement scale (CGI-I). Tolerability criteria were: incidence of withdrawal and incidence of AEs occurring in more than 5% of patients.
Results: Two trials were eligible for inclusion for PPX ($n = 689$) and three for RPR ($n = 931$). The direct meta-analysis, using random-effects model, confirmed superior efficacy for both treatments vs. Placebo measured as change on the IRLS (PPX: −5.5; 95% CI: −7.7; -3.2; RPR: −3.2; 95% CI: −4.3; −2.1) and CGI-I response (PPX: $OR = 3.0$; 95% CI: 2.1; 4.3; RPR: $OR = 2.0$; 95% CI: 1.5; 2.6). Compared to placebo only the incidence of nausea was found to be significantly higher for PPX ($P < 0.01$), whereas nausea, vomiting, dizziness and somnolence were significantly higher for RPR (all $P < 0.01$). The Bayesian indirect comparison showed a superior reduction on the IRLS for PPX vs. RPR of −2.3 points and had an $OR = 1.5$ for the CGI-I responders. The probability of superiority of PPX vs. RPR was 99% for the mean change in the IRLS score and 97% for the CGI-I responder rate.
Also the incidence of nausea ($OR = 0.37$), vomiting ($OR = 0.21$) and dizziness ($OR = 0.47$) were significantly lower under treatment with PPX vs. RPR with a probability of superiority of 99%, 98% and 95% respectively.
Conclusion: Results of the indirect meta-analysis showed with a probability ≥95% that PPX was superior to RPR for the mean change in IRLS and CGI-I response rate and lower incidence of nausea, vomiting and dizziness.

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Incontinence and insufficiency of following up and medical care of patients with spina bifida
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Introduction: An inquiry was done in a French population of patients in order to precise their difficulties and to bring on responses to their needs.
Methods: A questionnaire specially directed towards the problems of incontinence and sexuality was sent by mail and filled, either anonymously (14%) or not (86%) by themselves or with the help of their family. Only the completely filled questionnaires were taken into account. 165 patients aged from 2 to 61 years, have filled the questionnaire.
The questions examine also the neurologic and orthopaedic state, the number of surgical interventions, the mobility, the place and the means of life and more.
Results: Urinary incontinence was noted in 83% of the patients. The self-catheterization was only used in 63% of them, of whom 87% are dry between two catheterizations; some of these patients were improved by surgery. There is a lack of following up who explain the numerous complications, urinary infections, lithiasis, renal insufficiency leading to the dialysis. Sixty-three per cent of the patients have a faecal incontinence often associated with chronic constipation (98%) or acute diarrhoea (66%). The gastroenterological following up is poor: 13/165 have undergone a proctologic exploration. The sexuality is accomplished with difficulties: only 31% of the ladies and 35% of the men had sexual intercourses; the disorders of erection and ejaculation had as a consequence that only 5% of men have children (and 11% of the ladies) with healthy children.
Conclusion: A regular following up in all the concerned medical disciplines (and not only urologic, gastroenterologic, sexologic but also neurologic, orthopaedic and physiotherapy) would permit the spina bifida adults patients (about 20 000 patients) to have a better quality of life, to ovoid the most serious complications (renal insufficiency and neurologic complications with pain) and the daily difficulties bound to incontinence, constipation and sexual intercourses. This following up would have to be insured by regional and multidisciplinary clinics with a seal of quality, therefore labelled.

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Meta-analysis on survival data comparing docetaxel and vinca alcaloid in the first line treatment of non-small cell lung cancer
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Introduction: Taxanes and vinca alkaloids are commonly used in the first-line treatment of advanced NSCLC. We performed a meta-analysis assessing the efficacy and safety of docetaxel-based chemotherapy compared to vinorelbine- or vindesine-based chemotherapy in terms of overall survival and serious adverse events.
Methods: A systematic literature review of randomised clinical trials of docetaxel vs. vinca alkaloid chemotherapy in the first line treatment of advanced NSCLC was performed using MEDLINE, CANCERLIT, MEDSCAPE, Google Scholar, Cochrane Library, NIH RCT register, abstract books and meeting proceedings. All investigators and sponsors were contacted to supply study protocol, statistical and clinical study reports. The efficacy analysis was performed on an intention-to-treat basis. Analysis of survival was based on the pooling of individual logarithms of the hazard ratio (HR). Summary data were pooled by the inverse-variance weighting method. When the HR was missing and the life table was not available, a HR was derived from the number of deaths and the log-rank *P*-value using four decimals (1) Pooled odds ratio (OR) for safety outcomes were computed. A HR or OR less than 1 indicates that docetaxel is superior to vinca alkaloids. Heterogeneity was assessed using the Cochran Q-test.
Results: From eight potentially eligible trials, seven were selected ($n = 2867$). Docetaxel was administered with a platinum agent (three trials), with gemcitabine (two trials), or as monotherapy (two trials). Vinca alkaloid (vinorelbine [six trials]; vindesine [one trial]) was administered with cisplatin (six trials) or alone (one trial). The pooled estimate for overall survival showed an 11% improvement in favor of docetaxel (HR 0.89; 95% CI, 0.82–0.96; $P = 0.004$). Sensitivity analyses considering only vinorelbine as comparator or only the doublet regimens showed similar improvements. Grade 3 to 4 neutropenia and grade 3 to 4 serious adverse events were less frequent with docetaxel vs. vinca alkaloid-based regimens (OR 0.59; 95% CI, 0.38–0.89; $P = 0.013$ and OR 0.68; 95% CI 0.55–0.84; $P < 0.001$, respectively).
Conclusion: Meta-analysis on survival data based on hazard ratio rather than on median survival or mortality rate at 1 or 2 years is feasible when statistical study reports are available. The results of this meta-analysis support the positive benefit-to-risk ratio of docetaxel compared with vindesine or vinorelbine-based chemotherapy in the first-line treatment of advanced NSCLC.

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Readability and density of information in biomedical research: QuIP-5 study
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Introduction: This study was performed in order to compare the lexicosyntactic readability, and the information density in the informed consent forms used in biomedical research, in comparison with standard scientific texts dedicated to the general population. In addition, we studied whether there is a correlation between information readability and density.

Methods: Fifteen informed consent forms, six articles from (Sciences et Avenir) and six articles from (Sciences et Vie Junior) were analyzed. The lexicosyntactic readability was calculated using the Flesh score, and the information density using the number of information bits related to the number of words.
Results: The lexicosyntactic readability was lower in the informed consent forms (25, 17–32) compared with (Sciences et Avenir) (32, 29–38), but even higher in (Sciences et Vie Junior) (42, 38–57). Conversely, the information density was similar in (Sciences et Vie Junior) (0.24, 0.21–0.27) and the informed consent forms (0.24, 0.22–0.26), but higher in (Sciences et Avenir) (0.32, 0.26–0.38).
Conclusion: Informed consent forms are less readable, but paradoxically less dense than scientific papers dedicated to the general population. There is no correlation between density and readability.

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Rationale, design and methods of the OSCAR study: observational study on cognitive function and systolic blood pressure reduction in hypertensive patients

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Introduction: Data from several recent clinical trials have suggested a beneficial effect of antihypertensive medications on preservation of cognitive function. Eprosartan, an angiotensin type-1 receptor antagonist (ARA) with dual action on both pre- and postsynaptic angiotensin type 1 receptors, may be effective in the control of SBP and the prevention of cognitive decline.
Methods: The OSCAR study is an international longitudinal observational study with a duration of 6 months intended to examine the impact of eprosartan on cognitive function [assessed using the Mini-Mental State Examination (MMSE)] and control of systolic blood pressure (SBP) in a large international population of hypertensive patients managed in a standard primary care setting. A total of 100 000 hypertensive patients, above 50 years old and with SBP of >140 mmHg will be recruited by more than 20 000 primary care physicians in 27 countries. These patients will receive eprosartan 600 mg once a day for 6 months. The MMSE, will be performed at baseline, and after 6 months of treatment. After the first month of monotherapy, eprosartan treatment may, at the absolute discretion of individual investigators, be supplemented with other antihypertensive medications for the remainder of the study. The primary outcome indices are the mean relative change in MMSE score and the absolute change from baseline in SBP in the whole study population and in subsets of patients according to various factors among them: ethnicity, comorbidities (i.e. Target organ damage, diabetes), baseline cognitive level and baseline blood pressure level. The secondary objectives are to identify factors influencing SBP and MMSE changes. The OSCAR trial is the first international observational study focusing on MMSE in a wide international cohort of hypertensive patients.
Results: Recruitment began in January 2004 and as of 31 December 2005 a total of 37 597 subjects had been screened by 11 403 clinicians in 27 participating countries. Results are expected in 2007.
Conclusion: OSCAR represents an attempt, in a large number of countries, some of which have little tradition in this form of clinical research, to investigate the ability of an antihypertensive regimen to aid the preservation of cognitive capacity and function in patients with newly diagnosed hypertension. Further analysis will also allow comparisons' and analysis related to diversity of patients ethnic origin, healthcare systems in 'real life'. The results of this observational study may contribute to the identification of new outcome goals in hypertension.

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Patterns of drug consumption in a population of healthy subjects

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Introduction: Drug consumption is an important factor in health costs, and comes from both medical prescription and self-medication. Many drugs are easily used among young people, who consider them unexpensive and safe. However, no drug is costless, without side effects, and chronic drug intake can lead to various long-term consequences on health. The aim of our study was to evaluate drug consumption in a population of young healthy subjects.
Methods: We prospectively analysed drugs (other than contraceptive pills) taken by 95 healthy volunteers aged 18–40 (50 women, 45 men) included in a clinical trial evaluating the tolerance of a vaccine. The follow-up period was 59.9 ± 3.15 days. Treatments with immunomodulating agents were excluded, and non-steroidal anti-inflammatory drugs were limited to 14 consecutive days. Main outcome measures were per cent of subjects who took a drug, mean number of drugs taken, mean duration of treatment, class of treatments used. Results are given as mean ± Standard Error of the Mean (SEM). Comparisons were performed by Chi-square or Mann–Whitney test when appropriate.
Results: Drugs were taken by 55.7% of subjects (men: 40%, women: 70%, $P = 0.003$). Self-medication represented 84.3% of treatments. Among those treated, mean number of medications was 2.02 ± 0.19 (men: 1.72 ± 0.23, women: 2.17 ± 0.26, NS), and mean duration of treatment was 13.17 ± 2.90 days (men: 10.17 ± 3.92, women: 14.77 ± 3.94, NS). Therefore total treated time for these subjects was 22.0% of total follow-up time. Acetaminophen was the most frequent treatment in both men (48.4%) and women (51.3%). Among the 107 medications taken in the whole population, 11.4% were not taken appropriately when considering alleged indication and/or dosage (men: 11.5%, women: 11.3%, NS).
Conclusion: Although our population is composed of young subjects, considered healthy after a medical examination and included in a clinical trial, we detected a frequent use of medications. This use is significantly more frequent in women, and is essentially related to self-medication. In addition to raising concern about the risk of pharmacological interaction during clinical trials, our results question the attitude towards medication in our society.

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Benefit of early low dose ciclosporin on renal function in *de novo* cardiac transplant patients: preliminary analysis

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Introduction: Ciclosporin (CyA) has positively impacted on the outcome of cardiac transplantation; however CyA use is associated with chronic renal failure. In an effort to reduce exposure to CyA and possibly alleviate its nephrotoxic effects, we investigated the effect of early low dose CyA in association with mycophenolate mofetil (MMF) on serum creatinine levels during 12 months (M) of treatment in *de novo* heart transplant recipients.
Methods: In a multicenter, prospective French study, 95 *de novo* heart transplant patients receiving MMF and corticoids were randomized within 4 days after surgery to either *low dose* CyA (*LD,* $130 \leq T0 \leq -200$ µg/L $n = 48$) or *standard dose CyA* (*SD,* $200 \leq T0 \leq -300$ µg/L $n = 47$). Primary endpoint was the assessment of serum creatinine level. Secondary outcomes included the incidence and severity of acute rejection episodes. At M6, a preliminary analysis was performed on intent to treat population. A two level mixed model was used to compare creatinine evolution with a Peto adjusted significance level of 0.001 to preserve the 0.05 level for the final analysis at M12. Informed consent was obtained from all patients.
Results: *LD* and *SD* patients were comparable at baseline for all variables except for sex: *LD*-patients were principally men (76%) while sex was equally distributed in *SD* patients. Irrespectively of the CyA dose, there was a progressive decrease in creatinine levels during the first 50 days due to the transplantation: from 125 µmol to 101 µmol/L (*LD*) and from 128 µmol/L to 113 µmol/L (*SD*). Between Day 50 and M6 following the transplantation, creatinine values increased to 109 µmol/L (*LD) and 129* µmol/L *(SD),* with no significant difference between groups ($P = 0.075$). However, a trend toward a lower increase was found in favour to *LD* treatment. These trends were observed in average with a strong individual variability and should be confirmed with further analyses. No significant differences in acute rejection episodes, dialysis occurrence or death were observed between groups. Rejections occurred in 51% and 46% of patients in respectively LD and SD. One patient from the *LD* group and five patients from the *SD* group underwent dialysis. Infections were reported in one patient from *LD* group and two patients from *SD* group. One patient from the LD group and three patients from the *SD* group died.
Conclusion: These preliminary 6-M results show that a CyA dose reduction seems to slow down the progression of kidney disease, without evidence of increase in cardiac rejection compared to *SD* CyA dose. The 12-M final analysis will elucidate if this tendency can be confirmed.

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Predictive factors for thrombosis and major bleeding in an observational study including patients with heparin-induced-thrombocytopenia treated with lepirudin

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Introduction: Lepirudin, a direct thrombin inhibitor, is currently recommended in France for the treatment of heparin induced thrombocytopenia. Three prospective studies demonstrated that lepirudin, administered according to its recommended dosage, reduced the occurrence of thrombotic events by 90% but at the cost of increased bleeding risk. The aim of the study was to identify predictive factors for thrombosis and major bleeding in patients with heparin induced thrombocytopenia treated with lepirudin.
Methods: A retrospective observational study was conducted in France and Switzerland including all adult patients treated with lepirudin between 1997 and 2004. Clinical physicians and biologists working in the fields of thrombosis and haemostasis, pharmacists of private or public hospitals and French pharmacovigilance centers were contacted in order to identify the patients treated with lepirudin during the period study. Primary study outcomes were the occurrence of thrombotic and bleeding events while the patients were treated with lepirudin. The observation period lasted from the beginning of lepirudin treatment until hospital discharge. A central independent critical committee adjudicated all events as well as the diagnosis of heparin induced thrombocytopenia. Univariate and multivariate analysis were used to determine the factors associated with the occurrence of thrombotic and bleeding events.
Results: Among 219 patients treated with lepirudin, the critical event committee confirmed the diagnosis of heparin induced thrombocytopenia in 181 patients. The median age of the population was 67 years, 22% of the patients were at least 75 years old. Renal impairment (creatinine clearance less than 60 ml/min) was observed in 45% of patients. Fifty-nine per cent of patients received a bolus dose of lepirudin at treatment initiation and the mean lepirudine dose was 0.06 ± 0.04 mg/kg/h during a mean treatment period of 7.7 days. Twenty-five patients (13.5%) experienced a thrombotic event and 37 (20.4%) experienced a major bleeding. On multivariate analysis, only the administration of curative doses of heparin was a significant positive predictive factor for thrombosis during lepirudin treatment while renal impairment, duration of lepirudin treatment and a mean dose of lepirudin greater than 0.07 mg/kg/h were significant positive predictive factors for major bleeding with odds ratio of 13.20, 1.10 and 11.01 respectively.
Conclusion: The standard approved dosing of lepirudin for treatment of heparin induced thrombocytopenia is too high.

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Benefits and harms of induction immunosuppression in kidney transplantation: preliminary results

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Introduction: The use of induction therapies in kidney transplantation is controversial. Benefits on acute rejection have been demonstrated but it is not clear whether induction therapy improves long-term survival or graft loss. There is also some concern that induction treatment increases the risk of infection and malignancies.

Methods:
Objectives: The aim of this meta-analysis is to summarise long-term benefits and harms of three main induction therapies: anti lymphocyte/thymocyte globulins (ALG/ATG), anti CD3 antibodies (AB) and interleukin-2 receptor antibodies (Il2-RA).
Data sources: We searched Medline (1966-June 2006), Embase (1980-June 2006) and the Cochrane Library (issue 4 2006) for published trials. Authors of relevant articles and manufacturers of induction therapies were contacted; references lists and abstracts of congress were hand-searched.
Selection criteria: All randomised controlled trials comparing induction therapy with placebo or other therapies were included. Fixed and random effect models will be used to summarize relative risk for dichotomous outcomes with their 95% confidence intervals (CI).
Results: A total of 2021 articles and 55 abstracts of congress were identified. After screening of titles and abstracts, 1529 papers were excluded. We performed a full paper review of 436 articles. A total of 193 articles corresponding to 109 trials (including six on-going trials) were included. Forty two trials evaluated Il2-RA, 16 monoclonal anti CD3 AB, seven ALG/ATG and monoclonal anti CD3 AB, 39 ATG/ALG and 5 alentuzumab, ICAM 1 monoclonal AB, anti CD7 AB, anti LFA1 AB and BMA031.
Conclusion: Our preliminary results show that a large number of trials have been performed to evaluate efficacy and safety of induction therapies. Meta-analysis is a useful tool to evaluate the quality of these trials, to summarize their overall benefit/risk ratio and to explore the heterogeneity between their results. A subgroup analysis will be performed accounting for diabetes mellitus, high rejection risk (panel reactive antibodies positivity, HLA mismatch, cold ischemia time, delayed graft function) and type of donor (living or cadaveric).

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A randomized study to evaluate a positioning cushion for lumbar puncture in pediatric onco-hematology

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Introduction: Repeated invasive procedures in children with cancer or hematological diseases generate anxiety, fear, stress and pain. Lumbar punctures (LP) are commonly performed for diagnosis or treatment. Pain control is achieved with local or general anesthesia, and/or sedation. Muscle release, and quietness of a child during LP have a direct impact on the success of the LP. We have developed a positioning cushion for LP which allows the child to be relaxed in an adequate position, and to maintain this position throughout LP. This study was aimed at evaluating the benefit of the device on the technical quality of LPs, on pain, anxiety, post LP syndrome, and on the satisfaction of children, their parents and caregivers.
Methods: The study was a two-centre, open, randomized trial, with two parallel groups. Children aged 2–18 years undergoing a LP were eligible, if not included in the study before. Those who had used the cushion before, with a medical reason preventing the use of the cushion, those refusing or whose parents refused could not be included. Randomization was stratified by centre. Four cushion sizes were available for the age ranges: 2–6 years, 6–10 years, 10–15 and 15–18 years. The primary outcome was the rate of success, defined as a LP reaching its objective at the first attempt, without hemorrhage (RBC > 50/mm^3 in the CSF sample). Secondary outcomes included: the child's pain using a visual analogic scale (VAS), parents and caregiver perception of the child's pain (VAS); the children, parents, caregiver satisfaction; children cooperation using the 'Le Baron Scale', and the occurrence of a post LP syndrome.
Results: A total of 124 children were included, 62 per group: 'Cushion' and control groups. Characteristics of children at inclusion did not differ between groups. In the intention-to-treat analysis, there was a higher rate of success and fewer post LP syndromes with the cushion (67% vs. 57% and 15% vs. 24% respectively), but the difference was not significant. The result remained unchanged after adjustment on platelet number, and number of prior LPs. In children over 6 years, the difference between groups was statistically significant (58.5% vs. 41.5% without cushion, $P = 0.031$). There was also less pain in children (22.7 vs. 27.4 mm), and more satisfied children (84.4% vs. 75.0%) with the cushion, but the difference was not significant.
Conclusion: The cushion significantly improved the success rate in children over 6 years old, but not in smaller children. The reason might be that LPs are easier in younger children, or that the cushion needs to be adapted for smaller children. The positioningcushion for LPs is promising in children treated for cancer or hematological diseases. Further uses might be foreseen, in other child or adult diseases, or for spinal anesthesia.

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A computational model for the physiological analysis of septic shock

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Introduction: Septic shock remains the leading cause of death in intensive care units. Despite considerable research efforts, the mortality rate remains very high. Relevant knowledge has been obtained through multiple experimental and clinical studies. Given the complexity of the systems involved, a true systemic analysis of the underlying phenomena might help understanding the global pathophysiology of septic shock. Mathematical modeling would allow such analysis and may provide an original, affordable and highly promising tool to seek for more efficient therapeutic procedures. Septic shock is clinically perceived as a serious life-threatening condition, it results from the loss of regulation of vital physiologic functions under a particular interaction with an immunostimulating microbial agent. In the early stages of the evolution of sepsis, the nature of this interaction dictates almost completely the clinical outcome and the prognosis of the disease. However, once the intensity of the immunologic response derives in multiple acute reactions from several other systems, the probability of regaining systemic homeostasis decreases dramatically within only a few hours.
Methods: Taking into account the complexity that lies beneath this pathological process, we have set up a multidisciplinary team composed of physicians, mathematicians, immunologists and pharmacologists. Gathering and assessing clinical and biological data is the second step. Based on this knowledge, construction of a descriptive model containing systems and interactions will be possible. The descriptive model will be translated into Mathematical formulas. Another bibliographic search for existing *submodels* and model parameters values will be necessary. After that, we will be able to test the model performance and validate it on existing data. Thanks to a privileged collaboration with an intensive care unit, we have access to data, systematically collected for all the patients hospitalized in this unit.
Results: The development of this computational model is expected to empower the search of novel therapeutic targets for the treatment of septic-shock related multiple organ failure. *In silico* simulation, which can be performed several times, allows us to test therapeutic or pathophysiological hypothesis, providing predictions, which can be explored and confirmed with *in vitro* or *in vivo* studies. Furthermore, it will allow us to seek for novel and more reliable biomarkers that will increase the power of prognostic scores.
Conclusion: Septic shock is a good example of complex pathophysiological situation for which mathematical modeling can help us to integrate multiple parameters. Clinician expectation for such tool is choice and application optimization of therapeutic strategies.

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The ASOS survey, 2006

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Introduction: Since 2001, the Centres d'Evaluation et d'Information sur la Pharmacodépendance (CEIP) network conducts an annual survey on narcotic antalgics to describe characteristics of the population treated and utilization patterns, to assess if prescription recommendations are respected and to compare the evolution of prescriptions over time.
Methods: A sample of 500 community pharmacies was randomly selected in France. The survey was a 1-week study. Besides a questionnaire about their opinion on the secured prescription forms, participating pharmacists were asked to fill in a special form for each patient with a prescription form including a narcotic antalgic.
Results: In 2006, 139 pharmacies participated (27.8%); 30.2% of pharmacies delivered no narcotic antalgic during the study week. A total of 215 patients were included: 56.6% were females and 43.4% males; mean age was 63.7 years (median: 64.2, range: 19–98). The mean number of patients per pharmacy and per week was 1.55. In 6.5% of cases, the prescription was not written on a secured prescription form and in 21.9% of cases, the dosage was written in figures. The most frequently prescribed antalgics were transdermal fentanyl, followed by long-acting morphine sulphate and short-acting morphine sulphate. The treatment was newly instituted in 23.3% of cases. Indication for treatment was cancer (38.5%), rheumatologic disease (34.3%) and neurologic disease (8.4%). The pain was chronic in 76% of cases. Long-acting morphine sulphate was used as opiate maintenance treatment in 3.5% of patients treated with this antalgic.
Conclusion: Recommendations are not well respected. Compared with the preceding surveys, there is more new treatments, the proportion of indication for cancer is decreasing since 2004, particularly for fentanyl prescription.

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Subcutaneous adipose tissue lipolysis is mainly due to atrial natriuretic peptide in overweight men during exercise

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Introduction: To compare the mechanisms involved in lipolysis in lean and overweight men, we studied lipid mobilization in subcutaneous adipose tissue (SCAT) during exercise using microdialysis with a control probe and two probes for drug administration.
Methods: Subjects matched for age and physical fitness performed an exercise bout (35 min at 60% of their maximal oxygen consumption) under placebo or 5 mg tertatolol (a β-adrenergic antagonist).
Results: With placebo, exercise increased dialysate glycerol concentration (DGC) in both groups. Phentolamine added in the probe potentiated exercise-induced lipolysis only in overweight subjects. When phentolamine and propranolol were added together in the dialysis probe, DGC was reduced when compared to phentolamine alone in overweight men whereas a reduction of DGC occurred in lean men when compared with the control probe. After tertatolol administration, the increase in DGC during exercise was similar whatever the dialysis perfusate. Exercise increased catecholamine and ANP plasma levels after tertatolol administration. Compared to the control perfusate under placebo, lipolysis was reduced in lean but not in overweight men. In both groups, the DGC increase with phentolamine and propranolol correlated with plasma ANP. A correlation between the increase in DGC and the decrease in plasma insulin was only found in lean subjects. Changes in plasma NEFA, glycerol, insulin and catecholamine concentrations were similar in both groups.
Conclusion: Alpha-2-antilipolytic effect was observed in overweight men, Lipolysis in SCAT during exercise was not related to beta-adrenergic stimulation or to the decrease in plasma insulin but to the increase in plasma ANP concentrations.

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Effects of high doses of selenium in septic shock: a placebo-controlled, randomized, double-blind, phase II study

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Introduction: Sepsis is associated with the generation of oxygen free radicals and decreased selenium plasma concentrations. High doses of sodium selenite could

reduce inflammation by direct pro-oxidative effect and increase anti-oxidant cell capacities by selenium incorporation into selenoenzymes. We investigated the effects of high doses of selenium in septic shock patients.
Methods: This was a prospective, multicenter, placebo-controlled, randomized, double-blind study performed in patients fulfilling usual criteria for septic shock. Patients received, for 10 days, sodium selenite (4000 µg on the first day, 1000 µg daily on the nine following days) or matching placebo using continuous intravenous infusion. Primary endpoint was time to vasopressor therapy withdrawal. Duration of mechanical ventilation, mortality rates at intensive care unit (ICU) and hospital discharge, and at 7, 14 and 28 days from randomization, and adverse events were recorded.
Results: Sixty patients were included (placebo: 29; selenium: 31). Median time to vasopressor therapy withdrawal was 7 days in both groups [95% CI = (5–8) and (6–9) in placebo and selenium groups, respectively; log-rank: $P = 0.713$]. The duration of mechanical ventilation was 25 ± 43 and 34 ± 54 days in placebo and selenium groups, respectively ($P = 0.762$). Mortality rates at ICU and hospital discharge, and at 7, 14 and 28 days from randomization did not significantly differ between groups. Adverse events rates were similar in the two groups.
Conclusion: Continuous infusion of sodium selenite (4000 µg on the first day, 1000 µg daily on the nine following days) was well-tolerated but did not improve clinical outcome in septic shock patients.

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Contribution of the toxicological analysis of the hair in the diagnosis of the forensic deaths implying methadone

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Introduction: Our forensic institute observed an increase in the cases of death for which low blood methadone concentrations induce problems of interpretation. In this context, we wanted to know if the toxicological analysis of the hair, as chronic marker of exposure, could contribute to evaluate if patients are chronically methadone maintained or temporary users.
Methods: The methadone and his major metabolite EDDP (ethyl-dimethyl-diphenylpyrrolinium) were analyzed in 26 subjects' hair: these persons are outpatients in a substitution unit and in six methadone associated forensic deaths. Hair was segmented (first cm is recent period, second cm is old period), 20 mg of hair was washed, cut, incubated in diluted HCl, extracted (solid phase extraction, SPE) and analyzed in GC-MS with selected-ion monitoring.
Results: We developed a technique with a limit of detection to 1 ng/mg of hair. For the patients under methadone treatment, the average capillary methadone concentrations and EDDP are respectively about 30.12 (0.9–142.3) and 1.76 ng/mg (traces–7.2) of hair, for average dose administrated orally of 44.3 mg/day (5–120). An absence of correlation between administrated daily dose and the capillary concentration of methadone ($R = 0.49$) and of EDDP ($R = 0.19$) was confirmed. For the forensic cases, the determination of the presence or not of methadone, in the two segments of hair, made it possible to classify them in three groups: naive subjects, recent (beginning or reintroduction of treatment) or chronically consumers.
Conclusion: Hair analysis suggests a not usual methadone consumer profile in one forensic case, and gives more information than the sole post-mortem blood concentrations (<1000 µg/L). Moreover, systematic hair analysis was extended to the other opiates, for which phenomena of cross tolerance were described. This brings additional information which needs to be discussed with clinical data, forensic autopsies and information which may be obtained (heroine users, known no 'drug addict').

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Pharmacokinetic study of mycophenolate mofetil and design of a bayesian estimator based on a limited sampling strategy in patients with systemic lupus erythematosus

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Introduction: Area under the plasma concentration–time curve (AUC) monitoring of mycophenolic acid (MPA) has been developed for individual dose adjustment of Mycophenolate Mofetil (MMF) in allograft recipients. Since MMF has recently been approved for the treatment of Systemic Lupus Erythematosus (SLE), though not associated with other immunosuppressants, drug monitoring is also worth investigating in this population.
The objectives of this study were to estimate MPA pharmacokinetic (PK) characteristics in SLE. To test different PK models to fit plasma mycophenolic acid concentration-time profiles. To develop a maximum a posterior probability (MAP) Bayesian estimator of MPA PK parameters in these patients, based on a limited sampling strategy.
Methods: Twenty adult patients with SLE given a stable 1 g/day, 2 g/day or 3 g/day dose of MMF orally for at least 10 weeks were included in this study. MPA was measured by HPLC-UV (11 plasma measurements over 12 h post-dose per patient). Free MPA levels were measured by HPLC with fluorescence detection. Two different one-compartment models with first order elimination were tested to fit the data: one convoluted with a double gamma distribution to describe secondary concentrations peaks, and one convoluted with a triple gamma distribution to model a third, later peak. Nonlinear regression and MAP Bayesian estimation were performed using the MMF® program developed at Limoges University Hospital.
Results: A large interindividual variability of MPA concentration-time profiles was observed. The mean C_{max}, C_{min}, t_{max} and $AUC_{(0-12h)}$ were: 13.6 ± 8.4 µg/ml, 1.4 ± 1.2 µg/ml, 1.1 ± 1.2 h and 32.2 ± 17.1 µg h/ml, respectively. The mean free fraction of MPA was 1.7%. The one-compartment model with first order elimination convoluted with a triple gamma distribution best fitted the data. Accurate Bayesian estimates of AUC_{0-12h} were obtained using three blood samples collected at 40 min, 2 and 3 h, with $R^2 = 0.95$ between observed and estimated AUC_{0-12h} and with a difference less than 20% in 16/20 patients.
Conclusion: A particular PK model was built to accurately fit MPA blood concentration-time profiles after MMF oral dosing, which should allow MMF monitoring based on AUC_{0-12h} in SLE patients, provided the accurate Bayesian estimator developed. The predictive value of targeting one specific, or different AUC values on patients' outcome using this estimator in SLE will have to be demonstrated.

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Therapeutic monitoring of amykacine

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Introduction: Amikacin is an antibiotic of the group of the aminosides. Its bactericidal effect is concentration-dependent. It should be used with care because of its narrow therapeutic window and its oto and nephrotoxicity.
The aim of our study is to determinate the correlation between the posology of amikacin and the plasmatic concentration.
Methods: It's a retrospective study from December 2000 to October 2005. Amikacin plasmatic concentration were measured by fluorescence polarisation immunoassay (FPIA). Patients benefited from two plasma samples: through concentrations: immediately before amikacin infusion (0.4 < normal rate < 10 µg/ml) and peak concentration: 60 min following infusion (normal rate 20–35 µg/ml).
Results: We achieved 530 dosages (296 through concentrations and 234 peak concentration) in 152 different patients, 89 men and 63 women with a sex-ratio of 1, 41. Age varied from 3 days to 72 years with a middle age of 23.5 years. All patients received amikacin in a fractional way (two infusions per day). The middle posology was 15.33 mg/kg/day (1.25–37).
The posology was 15 mg/kg/day in 19 patients (57 dosages) whereas it was less than 15 mg/kg/day in 52 patients (256 dosages) and in 81 patients (183 dosages) it was >15 mg/kg/day.
Among the 296 through concentrations, 59 (19.93%) were ≤ 0.4 µg/ml, 200 dosages (67.5%) were between the range of 0.4 and 10 µg/ml and 37 dosages (12.5%) were >10 µg/ml.
Among the 234 peak concentrations, 127 dosages (54.2%) were <20 µg/ml, 89 dosages (38.09%) were between 20 and 35 µg/ml and 18 dosages (7.69%) were >35 µg/ml. Statistic analysis showed lack of correlation between, through and peak concentrations and between posology and plasmatic concentration.
Conclusion: Monitoring amikacin levels is highly recommended for the purpose of identifying and treating amikacin overdose and ensure appropriate and safe therapy.

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Oral absorption of ampicillin: role of the paracellular route vs. PepT1

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Introduction: Numerous studies with Caco-2 cell layers have emphasized the role of the PepT1 transporter in the absorption of β-lactam antibiotics, thus explaining the high oral bioavailability of many of them. Ampicillin has a bioavailability of 30–40% in animals and man, and its widespread use in farm animals may contribute to the increasing presence of antibiotic resistant organisms in the environment. The aim of this study was to characterise the mechanism of absorption of ampicillin in the small intestine with a view to its enhancement.
Methods: The transport of ampicillin across the intestinal mucosa was studied using the improved rat everted gut sac. The ampicillin was quantified by liquid chromatography with mass spectrometry detection. The sacs were incubated in different concentrations of ampicillin, at different pH, in the presence of PepT1 inhibitors and following the removal of the mucin layer. Glycylsarcosine was also used as a confirmed high affinity PepT1 substrate.
Results: Increasing concentrations of ampicillin failed to show the saturation characteristic of transporter-mediated absorption. There was no increase in transport at low pH nor any inhibition with competing antibiotics or glycylsarcosine. The removal of the mucin layer with reduced pH had no effect on the transport of ampicillin or glycylsarcosine. The transport of ampicillin was increased in the presence of ethylenediaminetetracetic acid, an established opener of the tight junctions and hence the paracellular route between the epithelial cells.
Conclusion: In the small intestine, the evidence suggest that PepT1 plays a minor role in the transport of ampicillin: the majority passes via the paracellular route. The near-absence of paracellular transport in Caco-2 monolayers means that they may be poor predictors of the transport of hydrophilic molecules that may transit via transporters and/or the paracellular route.

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Cost-effectiveness of mycophenolate mofetil monitoring and dose adjustment in kidney transplant recipients

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Introduction: The therapeutic Drug Monitoring (TDM) of immunosuppressive drugs in organ transplant recipients is recommended to optimize the ratio benefit/risk in the prevention of graft rejection. However, the cost-effectiveness of such a procedure has not been evaluated. As part of a clinical trial to validate the TDM of mycophenolate mofetil (MMF) in renal transplant patients over the first year post-

transplantation, we assessed the cost-effectiveness of MMF dose-adjustment based on mycophenolic acid (MPA) AUC monitoring, as compared to a fixed-dose regimen (2 g/day).
Methods: A randomized trial was carried out in 11 French centres and included patients receiving basiliximab, ciclosporin A, MMF and steroids: 65 patients received 2 g/day MMF ('fixed dose' FD group), while 65 patients received an MMF dose adjusted on MPA AUC_{0-12h} ('concentration controlled' CC group). The principal clinical outcome was a composite criterion, called treatment failure (death, graft loss, acute rejection and MMF discontinuation). Secondary criteria were the incidences of adverse drug events (ADE) and acute rejection. Economic data were collected retrospectively and analyzed from the payer perspective. The direct medical costs were all hospitalisations, consultations, medications, TDM and complementary diagnostic tests over the first year post-transplant; and direct non medical costs were patients' transports. Incremental analysis was conducted: the ratios of the difference in costs to the difference in one or the other effectiveness criteria between the CC group and the FD group (i.e. Cost to avoid one treatment failure, one acute rejection episode with MMF TDM) were calculated.
Results: The incidence of treatment failures was to 29.2% (CC group) vs. 47.7% (FD group) ($P = 0.03$). This difference was mainly due to a difference in clinical acute rejection rates (12.3% vs. 30.8%, $P = 0.01$), while the incidence of ADE was slightly but not significantly higher in the CC group (97% vs. 91%, $P = 0.3$). The cost per patient was 49765 euros (CC group) vs. 48942 euros (FD group) ($P = 0.5$); the mean cost of the MMF TDM procedure was 998.3 ± 342 euros. The incremental cost-effectiveness ratio was 2891 euros to avoid one treatment failure and 2891 euros to avoid one acute rejection episode. Sensitivity analysis and boostrap analysis of costs showed that these estimates were not robust.
Conclusion: In the first year after kidney transplantation, TDM MMF significantly decreases the incidence of treatment failures and acute rejection rate as compared to a fixed-dose, without a significant increase of ADE. This study shows that this strategy is not sensibly nor statistically more costly.

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Therapeutic drug monitoring of tipranavir: about two cases
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Introduction: Tipranavir is a nonpeptidic protease inhibitor (NPPI) with a resistance profile distinct from that of other currently available protease inhibitors (PIs). Concomitant administration of low-dose ritonavir significantly increases tipranavir plasma concentrations; therefore, the approved dose of tipranavir is 500 mg with ritonavir 200 mg, twice daily (1). Severe adverse effects (hepatotoxicity and lipid abnormalities) request close monitoring. Hepatotoxicity seems to be in relation with overdosage of tipranavir. So, therapeutic drug monitoring may be helpful in optimizing outcomes. In order to assay tipranavir concentrations we validated an RP-HPLC method and applied it to analyse two cases of hepatotoxicity described for patients treated with tipranavir–ritonavir.
Methods: A method previously described for the assay of 10 IPs can be also conveniently used, with minor gradient program adjustment, for the determination of tipranavir in human plasma. (2) We used a solid phase extraction method coupled to an online injection for RP-HPLC quantification and validated its use to determine simultaneously plasma levels of 11 molecules in a single run. Pretreatment of 1 mL of plasma sample spiked with an internal standard (carbamazepine) was made by a solid phase cartridge (Waters Oasis HLB®) using an ASPEC®automate (GILSON®). This system allows injecting directly samples on line, without further step of experiments. Liquid chromatography was performed using a Symmetry C18 reverse phase column (Waters®) and gradient elution for 40 min. Detection and identification are optimized with a photodiode array detector. Quantification was realised at 210 and 240 nm.
Results: All drugs were well separated. Detection by photodiode array detector allowed spectral identification of these 11 drugs in the range of concentrations: 100 –30 000 ng/mL. Calibration curves for each drug were linear in this range to straddle therapeutic concentrations. Efficiencies of extraction (%) were calculated for these 11 molecules at six concentrations: 75% for ritonavir and more than 90% for most of the other drugs. Coefficients of variation were less than 15% for all drugs. We confirmed by assays that patients treated by tipranavir/ritonavir (500/200) twice daily with hepatic disorders (elevation of ALT/AST and hepatitis) presented overdosage of tipranavir (respectively 11 0071 and 12 0089 ng/ml).These ALT/AST elevations can be attributed to tipranavir itself, or maybe to the ritonavir used to boost it.
Conclusion: The combination of solid phase extraction and on line HPLC injection for identification and quantification of 11 drugs allows to minimize manipulations and to save time during the process of analysis of several samples.
Maintain of plasma level of tipranavir in the therapeutic range and adaptation posology seems to be necessary to explain and avoid adverse side-effects.

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An *in vitro* method to study drug absorption by the intestine of knockout mice
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Introduction: Several *in vitro* methods have been used to study drug absorption by rat intestine (everted sacs, using chambers, perfused segments). However, genetically modified rats are not available, whereas knockout (KO) mice are valuable tools, and some are available that lack specific transporters relevant to drug absorption. The most important are those lacking efflux transporters, such as P-glycoprotein (P-gp), which transports drugs out of intestinal cells and reduces net absorption. We have adapted the everted gut sac method to use with mice, tested it with normal mice, and studied the absorption of the P-gp substrate digoxin in P-gp knockout mice.
Methods: The small intestine was rapidly removed from the mouse, rinsed through with saline and immediately placed in warm (37°C) oxygenated tissue culture medium. It was gently everted over a fine glass rod, filled with oxygenated medium, and divided into 12–13 sacs sealed with silk. Sacs were incubated with appropriate radiolabelled marker molecules for different times and the contents then counted to obtain the net absorption.
Results: Markers for transcellular (antipyrine, testosterone) and paracellular (mannitol) transport showed linear kinetics for up to 45 mins, indicating good tissue viability. The relationship between the absorption rates of the compounds was similar to that found with rat gut sacs. The absorption of the P-gp substrate, digoxin, was measured over 45 min in normal and P-gp knockout mice. In both cases transport was linear, but the rate was over three times greater with intestine from knockout mice, showing that P-gp was important in limiting the absorption of digoxin by the normal intestine.
Conclusion: We have developed a simple but precise method for measuring drug absorption in mouse intestine *in vitro*. By using knockout mice, the role of transporters in drug transport processes can be rapidly evaluated.

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Impact of nevirapine or efavirenz coadministration and fosamprenavir daily regimen on ritonavir-boosted amprenavir pharmacokinetics in HIV infected patients
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Introduction: The influence of nevirapine or efavirenz (which are considered as inducers of protease inhibitors metabolism) coadministration and the type of fosamprenavir daily regimen (once daily vs. twice daily) on amprenavir pharmacokinetic was investigated in HIV infected patients.
Methods: A population pharmacokinetic analysis (covariates: nevirapine or efavirenz coadministration and the type of fosamprenavir daily regimen) was performed with a population of 79 patients [group A – 46 patients: fosamprenavir/ritonavir (700 mg/100 mg twice daily) associated with nucleoside/nucleotide reverse transcriptase inhibitors ±enfuvirtide; group B – 18 patients: fosamprenavir/ritonavir (1400 mg/200 mg once daily) associated with nucleoside reverse transcriptase inhibitors; group C – 10 patients fosamprenavir/ritonavir (700 mg/100 mg twice daily) associated with nevirapine ±nucleoside reverse transcriptase inhibitors ±enfuvirtide; group D – five patients fosamprenavir/ritonavir (700 mg/100 mg twice daily) associated with efavirenz ±nucleoside reverse transcriptase inhibitors ±enfuvirtide].
Results: No significant influence of covariates was found. However, a decrease of amprenavir trough plasma concentration was observed in the group B in comparison with the group A: 1.78 ± 0.97 mg/L vs. 1.09 ± 0.51 mg/L ($P < 0.05$).
Conclusion: When combined with efavirenz or nevirapine, fosamprenavir/ritonavir should be used at standard doses of 700 mg/100 mg twice daily. Once daily fosamprenavir/ritonavir led to lower amprenavir plasma concentration than those which could be obtained with a twice daily regimen.

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Abstract withdrawn

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Comparison of the new AxSYM® ciclosporin assay with high performance liquid chromatography (HPLC)
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Introduction: Besides its narrow therapeutic range, ciclosporin A has marked inter-and intra individual variations in biovailability and pharmacokinetics. Therefore, reliable monitoring assays and adequate ciclosporin A concentrations are crucial. Historically, methods to monitor blood levels of ciclosporin A can be divided in to high performance liquid chromatography assays and immunoassays. These assays differ in their specificity, imprecision and their analytical performance characteristics
The aim of this study was prospective, it is to compare the new Abbott AxSYM® (fluorescence polarisation immunoassays (FPIA) with monoclonal antibodies) ciclosporin A assay with high performance liquid chromatography used as reference method for determining blood ciclosporin A concentrations.
Methods: We compared 460 blood samples withdrawn from 100 hospitalised patients from February to Junw 2003. Every sample was analysed by the two methods. Comparison between the two assays was performed using ANOVA 1 test, linear regression analysis was also performed to determine if any correlation existed between them.
Results: Statistical analysis shows a good correlation ($r = 0.9865$) between the results of two analyses. ANOVA test used to compare the mean trough ciclosporin A concentration measured by both methods has shown a P value <0.05 that prove a non significant difference between the two assays.
The immunoassays yield slightly higher concentrations than done by high performance liquid chromatography (22.5%).
Conclusion: Despite the cross reactivity that happened between ciclosporin A and their metabolites, our result showed a good correlation between the two assays.
Based on these result we concluded that AxYM® is a precise method for measuring ciclosporin A and offers similar concentrations to those obtained by high performance liquid chromatography.

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Population pharmacokinetic analysis of ultrafilterable ciplatin after peroperative intraperitoneal administration

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Introduction: Ovarian cancer is the leading cause of gynecological cancer-related death in western countries. A recent additional treatment strategy might be based on intraperitoneal chemotherapy. Using such method, we previously described pharmacokinetic parameters of platinum with a 2-h intraoperative IP treatment and established that IP platin concentration required for cytotoxicity was insufficient. Here are presented a population pharmacokinetic analysis obtained after two consecutive one-h IP administrations of 30 mg/L of cisplatin.

Methods: Twenty-eight patients with advanced epithelial cancer classified FIGO stage IIIC underwent a debulking surgery during which the intraoperative intraperitoneal chemotherapy with cisplatin was performed (two one-hour-'bath' with 30 mg/L of cisplatin). Blood and IP samples were obtained at 1, 30, 59 min during each 'bath' then only blood sample 4, 6, 8, 16 and 24 h after treatment. All samples were ultrafiltered (Uf) and determined with ICP-MS. The population pharmacokinetic study was conducted using the nonlinear mixed effects model software, NONMEM, and the model was assessed using bootstrap for the estimation of standard errors and visual predictive check for model evaluation.

Results: A two-compartment open model with first-order elimination best fitted the data. The inter-individual variability was best estimated using the multiplicative model, while the intra-individual variability model was best described by an additive model associated with a proportional error model only linked to the compartment associated with the IP concentrations. The mean pharmacokinetic parameter were of 4.60 L and 9.19 L/h for the distribution volume and the peritoneal clearance for the compartment associated with the IP concentrations; and 26.5 L and 31.6 L/h for the distribution volume and the elimination clearance of the central compartment. The mean constant transfer between central compartment and 'peritoneal' compartment was of 0.42 h^{-1}. Among the covariables tested (age, weight, body surface area, height, serum creatinine, creatinine clearance, serum total proteins and peritoneal total proteins), an association between weight and the distribution volume of the 'peritoneal' compartment was observed, leading to a decrease of inter-individual variability from 25.2% to 19.8%.

Conclusion: This study allows to satisfactory describe both intraperitoneal and serum ultrafilterable platinum concentration with the same model. The elaboration of another model with total platinum is currently ongoing. These models will be used to develop a limited sampled strategy for the determination of patient's exposure.

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Combined inhibition of monoamine oxidase and semicarbazide-sensitive amine oxidase reduces food intake and body weight gain in obese rats

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Introduction: Aminoguanidine, a drug proposed for the prophylaxis of diabetic vascular complications, is not only able to inhibit NO synthase and to prevent protein glycation, but also inhibits semicarbazide-sensitive amine oxidase (SSAO). Since adipocytes highly express SSAO together with monoamine oxidases (MAO), and since their substrates have been shown to exert insulin-like effects, it was of interest to test the influence of their inhibitors on obesity. The aim of this work was to investigate whether chronic SSAO blockade by aminoguanidine or the combined inhibition of SSAO and MAO by irreversible inhibitors could influence glucose tolerance or adipose tissue deposition in the hyperinsulinic obese Zucker rat.

Methods: Aminoguanidine was i.p. administered at 270 μmol/kg/day for 3 weeks an 9-wk old obese rats, semicarbazide (S, 36, 100 or 300 μmol/kg/day) was given alone or in combination with pargyline (P, 20 or 61 μmol/kg/day) for 3 to 4 weeks.

Results: Aminoguanidine-treated rats lost their SSAO activity in adipose tissues and cerebral vessels while MAO was unmodified in liver. The treatment did not change body weight gain of obese rats, did not improve their hyperinsulinemic state and slightly reduced subcutaneous fat deposition. P+S treatments inhibited quite completely both MAO and SSAO in any tissue. Although without clear effect when administered separately, P and S led to a 12–16% reduction of food intake when given in combination (P+S). Despite a similar decrease in body weight gain between P+S treated-rats and pair-fed controls, inhibition of fat deposition was greater in the rats receiving inhibitors. Chronic treatments with MAO and SSAO inhibitors did not modify glucose tolerance. Adipocytes from P+S-treated rats responded to insulin activation of glucose uptake as control but became unresponsive to the insulin-like actions of benzylamine (SSAO substrate) or tyramine (MAO substrate) plus vanadate, while those from AG-treated rats lose only their response to benzylamine.

Conclusion: These observations indicate that SSAO inhibition is insufficient to impair fat deposition while combined inhibition of MAO and SSAO reduces food intake, likely by altering the fate of neurotransmitters involved in appetite regulation, and limits adipose tissue development probably by preventing insulin-like actions of biogenic or alimentary amines.

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Effect of CYP2C19 polymorphism on nelfinavir to M8 biotransformation

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Introduction: To evaluate the effect of CYP2C19 polymorphism on nelfinavir and M8 pharmacokinetic variability in HIV infected patients.

Methods: One hundred and twenty nelfinavir and 119 M8 concentrations were measured in 34 naive-patients enrolled in the COPHAR 2-ANRS 111 trial. Two weeks after initiating the treatment, four blood samples were taken at $T = 1, 3, 6$ and 12 h. Genotyping for CYP3A4, 3A5, 2C19 and MDR1 (exon 21 and 26) was performed. A population pharmacokinetic model was developed in order to describe nelfinavir and M8 concentration time-courses and estimate inter-patient variabilities. The influence of individual characteristics and genotypes were tested in the population model using a likelihood ratio test.

Results: A one-compartment model with first-order absorption, elimination and metabolism to M8 best described nelfinavir data. M8 was modelled by an additional compartment. Mean pharmacokinetic estimates and the corresponding inter-subject variabilities (%) were: absorption rate 0.17 h^{-1} (99%), absorption lag time 0.82 h, apparent nelfinavir elimination clearance 51.6 L/h (49%), apparent volume of distribution 191 L, M8 elimination rate constant 1.76 h^{-1} and the apparent formation clearance to M8 for a M8 distribution volume fixed to one, (CL_m/V_m) 0.39 h^{-1} (59%). This CL_m/V_m was divided by 1.98 in patients mutated for CYP2C19 gene. With respect to link between pharmacokinetic and short term metabolic toxicity, nelfinavir C_{res} and AUC were positively correlated to glycemia variation ($P = 0.03$, $P = 0.02$) and AUC to triglycerides variation ($P = 0.04$).

Conclusion: Patients with an *1/*2 + *2/*2 genotype for CYP2C19 had a nelfinavir to M8 biotransformation divided by 2 compared to *1/*1 patients. In these antiretroviral-experienced, Pi-naïve patients, efficacy could not be related to concentrations but triglycerides and glycemia increased with nelfinavir exposure.

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Pharmacokinetics of voriconazole during high volume hemofiltration

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Introduction: Voriconazole (VRC) is a new triazole antifungal agent effective against invasive *Aspergillus* and *Candida* infections. In a recent study, authors recommended administration of standard dose of voriconazole during renal substitution by continuous venovenous hemodiafiltration (CVVHDF). A way of increasing the dose of dialysis is to use continuous high-volume hemofiltration (CHVH) which employs ultrafiltration (UF) volumes greater than 35 mL/h/kg. But in hemofiltration, the convective clearance being directly dependent on the flow of UF, the elimination of drugs is increased.

Methods: It's a prospective study. We measured VRC plasma concentration by HPLC reverse phase with a limit of quantification 0.1 mg/L. We have studied changes of VRC elimination during CHVH among two patients.

Patient 1: a 52-year-old, 70 kg woman, with history of Crohn's disease was transferred in our unit to fifth post-operative day of peritonitis by intestine perforation. The patient received treatment by intravenous VRC at conventional dosage (2 h perfusion of 6 mg/kg twice day as loading dose followed by 4 mg/kg every 12 h). The analysis was carried twice: first on the second loading dose of 400 mg during CHVH with pre-dilution flow and on the third dose of 300 mg at balance condition during CHVH with post-dilution flow.

Patient 2: a 49-year-old, 80 kg woman was admitted for a septic shock. She was treated by intravenous VRC at conventional dosage. The analysis was carried on the second loading dose of 480 mg during CHVH with 150 mL/min blood flow, 350 mL/H UF subtraction and 4000 ml/h pre-dilution flow and on the third dose of 320 mg at balance condition during CHVH with 200 mL/min blood flow, 200 mL/H UF subtraction and 4000 ml/h post-dilution flow.

Samples were drawn simultaneous from CHVH arterial, venous and ultrafiltration lines before administration and at 60, 120, 140, 160, 180, 360, 540 and 720 min. Total volume of ultrafiltration was measured and samples collected for determination of total VRC dose eliminated during 12 h. The sieving coefficient was calculated by the Coltonformula: $Sc = 2\ Cuf/(Ca + Cv)$.

Results: First, we have found that the elimination of the VRC in CHVH is mainly increased well above 6% previously described. These results are the consequence of the increase in the UF flow of which depends directly on the convective clearance.

Conclusion: In predilution CHVF, the percentage of VRC elimination remains insufficient to recommend an adaptation of dose. But in post-dilution CHVF, this percentage is significantly increased above 40%.

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Acute intoxication by tramadol: interest of pharmacokinetics follow up during the critical phase of survival

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Introduction: Tramadol (TMD) is a synthetic opioid used per os. In the WHO classification it is a group IIa analgesic. Its mechanism of action is double: (i) like other opiates it binds to the various types of opioid receptors with a low affinity (compared to morphine the Ki are 2.4 μM and 0.62 nM, respectively); one of its métabolites [O-desmethyl-Tramadol (ODT)] displays similar efficacy and affinity. (ii) TMD is an inhibitor of Norepinephrine and Serotonin reuptake. This double tropism explains the symptoms observed after acute intoxication including excitement, mydriasis, convulsion and then cardiac and respiratory failures. We report here a case of an acute intoxication – 10 g of TMD – with a final good outcome after a prolonged extra-corporeal circulatory assistance (ECCA). At admission Glasgow was three and the tachycardie became asystolia requiring an ECCA for 7 days.

Methods: DMT and ODT where measured in blood after admission (corresponding to 10th hour post intoxication), 12 h later and every day until D8. HPLC-MS/MS was used and the results display a very closed parallelism between TMD and ODT.

Results: At admission the peak of TMD was 25 mg/L (about 125 time the therapeutic level) while ODT increased until the 24th hour. Then we observed T1/2 of 19 and 23 h for TMD and ODT respectively during the 6 days of ECCA. Finally the T1/2 became normal when the cardiac function normalized. Liver and muscle enzymes normalized simultaneously the cardiac output increased.

Conclusion: Compared to main cases of the literature the survival was unexpected. Previously no-one benefited of ECCA which has been efficient during the critical phase of cardiac sideration. Fortunately the patient completely recovered heart, liver and kidney functions. Ten days after intoxication he became independent and 3 months later he had a normal social life again. The double interest of blood TMD monitoring is (i) to confirm fast the lethal dose ingested and (ii) to follow the cardiac recovery. The absence of after effects is also encouraging to resuscitation with ECCA in case of massive Tramadol intoxication.

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***In vitro* models to complete hERG channel assay in preclinical cardiac safety pharmacological studies**

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Introduction: Recommendations of the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use concerning cardiovascular adverse effects are focused on hERG channel assay and QT prolongation. However, cardiac adverse effects induced by pharmaceutical compounds can not be exclusively attributed to hERG channels block and QT prolongation. Indeed, sodium channels block has also been linked with the induction of severe arrhythmias. Thus, the aim of the present study was to investigate the effects of two class I antiarrhythmic drugs, flecainide and quinidine, known to block both $Na_{V1.5}$ and hERG currents, on the action potential parameters in rabbit atria and Purkinje fibres. We have also determined the effects of the sodium channel blockade properties of these compounds on the atrial conduction speed.

Methods: The effects of quinidine (10 μmol/L) and flecainide (10 μmol/L) have been first investigated on hERG and $Na_{V1.5}$ channels, both transfected in HEK-293 cells using the patch-clamp technique. Moreover, effects of quinidine and flecainide on AP parameters and atrial conduction speed have been determined using standard microelectrode technique.

Results: The 92% hERG inhibition induced by quinidine led to reverse-use increases in action potential duration (APD_{90}: +15% and +69% at 1 and 0.2 Hz) and in action potential triangulation ($APD_{90–40}$: +73% and +152% at 1 and 0.2 Hz) in Purkinje fibres. The effects of quinidine on triangulation were less marked in atria (+26% at 1 Hz). On the contrary, the 85% hERG inhibition induced by flecainide had no effects on both action potential duration and triangulation in Purkinje fibres and atria. The 32% $Na_{V1.5}$ block induced by quinidine led to a decrease in depolarization rate (dV/dt_{max}) more marked in atrial action potential (-20%) compared to Purkinje fibres (−13%) at 1 Hz. In the same way, flecainide blocked the $Na_{V1.5}$ current by 73% and decreased dV/dt_{max} by 22 and 32% in Purkinje fibres and atria at 1 Hz. Finally, quinidine and flecainide decreased the atrial conduction speed by 15 and 31% at 1 Hz.

Conclusion: As previously described with flecainide, the hERG channel inhibition should not be considered as a useful single assay in cardiac safety pharmacological studies since false-positive results may be observed. Action potentials of rabbit Purkinje fibres can be considered as a good *in vitro* model for detection of reverse-use action potential prolongation and triangulation whereas rabbit atria can be considered as a useful model for detection of sodium channel block, linked with decreases in depolarization rate and atrial conduction speed.

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Compliance of slow acting drugs in osteoarthritis in medical general practice

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Introduction: Slow acting drugs for osteoarthritis are largely used and were the subject of many clinical evaluations in the osteoarthritis of the lower limbs and the hand but data are missing about slow acting drugs compliance in osteoarthritis.

Methods: National transverse descriptive epidemiologic investigation in general medical practice: 991 general practitioners described 991 ambulatory patients suffering from osteoarthritis and were asked location of osteoarthritis, evolutionary moment and duration of the treatment, methods of administration, evaluation of the compliance by GP's using a VAS, causes of poor compliance, methods to improve the compliance.

Results:

- The treatment by slow acting drugs in osteoarthritis is generally prescribed like treatment of maintenance (42.7%). It is associated with oral NSAID in 58.4% of the cases and analgesics of level I in 57.5% of the cases. The duration of the treatment is higher than 3 months in 42% of the cases, continuously (52.2%), by cure (42%) at a rate of 2.6 cures/year. It is continued between 2 and 5 years in 45.4% of the cases.
- Functional rehabilitation is associated with pharmacological treatments in 78.5% of the cases, and a reducing diet in 60.3% of the cases.
- The observance of the treatment by slow acting drugs in osteoarthritis and the antalgic treatments is considered to be good or good enough mainly for 81% of the general practitioners. That of local NSAID treatments and the nonpharmacological treatments is judged less favorably (60.2% and 44.2% respectively).
- For slow acting drugs in osteoarthritis and analgesics, compliance is significantly better compared to the whole of the other treatments ($P < 0.001$).
- For the local NSAID and nonpharmacological treatments, compliance is significantly worse compared to the whole of the other treatments ($P < 0.001$).
- The called upon reasons of bad observance are generally the lapse of memory of the treatment (54%), the fear of the iatrogenic effects (40%) or habituation (36%), an age of the patients higher than 75 years (31%).
- To improve the compliance, the general practitioners mainly recommend better information on osteoarthritis and its treatments as well as an easier presentation of the drugs. About half of them (47.4%) admit nevertheless sufficiently not spending time with their patients.

Conclusion: The observance of the treatment by slow acting drugs in osteoarthritis and the antalgic treatments is considered to be good or good enough mainly for 81% of the general practitioners. That of local NSAID treatments and the nonpharmacological treatments is judged less favorably. For slow acting drugs in osteoarthritis and analgesics, compliance is significantly better compared to the whole of the other treatments.

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Lopinavir population pharmacokinetics on HIV-infected adults – body weight and co-treatments influence

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Introduction: Lopinavir is a protease inhibitor (PI) used in HIV-infection treatment with a current recommended dosing regimen of 400 mg (with 100 mg ritonavir) BID. The aim of our study was to develop a population pharmacokinetic model highlighting the possible influence of co-variates such as bodyweight and other anti-retroviral treatments. Such a model could be useful to evaluate the current recommended dose with regard to lopinavir target plasma concentrations.

Methods: The pharmacokinetics of lopinavir were investigated using a population approach performed with NONMEM on 709 HIV-1-infected patients (1275 samples). Individual Bayesian estimates of lopinavir pharmacokinetic parameters were used to calculate the minimal concentrations obtained with various dosage regimens. The probability to achieve previously determined target concentrations in PI-naïve (i.e. 3 mg/L) and PI-pretreated (4.7 and 6 mg/L) patients was calculated for each regimen. Influence of bodyweight on the probability to achieve these target concentrations was evaluated by logistic regression.

Results: Lopinavir pharmacokinetics was well described by a one-compartment model, with typical population estimates (interindividual variability %) of 4.5 L/h (34.1%) and 33.5 L (43.5%) for apparent clearance and distribution volume, respectively. Bodyweight, concomitant treatments with nevirapine, efavirenz, and amprenavir were found to have an influence on lopinavir pharmacokinetics. The 400 mg BID regimen enables 94% of the population to reach a minimal concentration 12 h after last intake of 3 mg/L, but enables only 79% and 45% of the population to reach a 4 and 5.7 mg/L concentration. Furthermore, a 10 kg increase in bodyweight significantly decreased the probability to reach target concentrations for PI-pretreated patients when using a 400 mg BID regimen (odds ratios of 0.57 and 0.56 with $P < 0.0001$).

Conclusion: The 400 mg lopinavir-100 mg ritonavir BID regimen is efficient to reach a minimal concentration of 3 mg/L for PI-naïve patient but may not be suitable for PI pretreated patients. Bodyweight has an influence on the minimal 12 h after intake concentration and should be taken into account when initiating a therapy to a pretreated patient.

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Clinical value of azathioprine metobolites assessement in IBD patients: a Tunisian experience based on a cohort 76 patients

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Introduction: Azathioprine (AZA) is currently the principal maintenance therapy for inflammatory bowel diseases (IBD). However, its effectiveness and tolerance might vary from a patient to another. After intestinal absorption, AZA is quickly metabolized into 6-mercaptopurine (6-MP). Activation of 6-MP occurs via hypoxanthine-guanine phosphoribosyltransferase (HGPRT) and a series of multi-enzymatic processes involving kinases to form 6-thioguanine nucleotides (6-TGNs) as major metabolites. The cytotoxicity of azathioprine is due, in part, to the incorporation of 6-TGN into DNA. 6-MP undergoes two major inactivation routes. One is thiol methylation, which is catalyzedby the enzyme thiopurine S-methyl-transferase (TPMT), to form the inactive metabolite methyl-6-MP (6-MeMP). The aims of our study was monitor AZA, and its major metabolites, blood levels and to try to find a correlation between 6-TGN blood concentration and therapeutic response to AZA in our cohort of IBD patients.

Methods: Patients with Crohn's disease (CD) or Ulcerative Colitis (UC) treated with AZA were included in the study. A High Performance Liquid Chromatography (HPLC) method was used to measure AZA and its metabolites in the plasma of CD and UC patients

Results: Seventy-six patients were included in the study, 54 with CD and 22 with UC. Among the 76 patients 42 were men and 34 women and the mean age was 31 years. The mean concentrations for 6-TGN and 6-MeMPN were 326 pmol/8 10^8 RBC and 434.51 pmol/8 10^8 RBC respectively. In our cohort of patients 6-TGN synthesis was the main AZA metabolic pathway for 60% of the patients whereas for 40% of the patients it was 6-MeMP production.

Xe observed a positive correlation between a 6-TGN cut-off value of 300 pmol/8 10^8 RBC and clinical response to AZA.

Conclusion: HPLC monitoring of azathioprine metabolites allowed us to find that 6-TGN was the main metabolic pathway in our cohort of Tunisian IBD patients. We also defined a 300 pmol/8 10^8 RBC 6-TGN value as a predictor of clinical response to AZA. These values should be recommended in clinical practice to monitor IBD patients, in particular those not responding to AZA treatment despite an appropriate weight-based daily dose.

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Concentration-effect relationship of bevacizumab in a hemodialysed patient with kidney cancer

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Introduction: Renal Cell Carcinoma (RCC) is one of the most challenging malignancies to treat. Bevacizumab, a humanised monoclonal antibody against vascular endothelial growth factor (VEGF) has shown a substantial antitumour effect in RCC. We report a pharmacokinetic and concentration-effect study of bevacizumab in a hemodialyzed patient.

Methods: A 23-year-old Caucasian male had been treated since age three for kidney clear cell sarcoma. After bilateral nephrectomy, he was treated by hemodialysis thrice a week. Because of the occurrence of metastasis, bevacizumab was instituted at a dose of 5 mg/kg every 2 weeks. This led to a decrease in tumour mass and an increase in quality of life. To assess the patient's exposure, a pharmacokinetic-pharmacodynamic (PK-PD) study was performed over a dosing interval. Blood samples were collected just before and 1, 2, 3, 4, 5, 6, 12, 18 and 24 h after infusion, and just before, during and immediately after each dialysis session. Both VEGF and bevacizumab serum concentrations were determined by ELISA methods. Bevacizumab pharmacokinetic and concentration-effect relationship were described by a two-compartment model and an indirect response model, respectively.

Results: Estimated pharmacokinetic parameters were similar to those reported in subjects with normal renal function receiving 10 mg/kg every 15 days, with a clearance of 0.122 mL/min and a central volume of distribution of 2.5 L. Bevacizumab pharmacokinetics was not modified by hemodialysis since concentra-

tions in the dialysate were below the detection limit. A decrease in VEGF concentrations was observed with values back to baseline after 4 days. These changes were satisfactorily described by an indirect response model with inhibition of VEGF production. The bevacizumab concentration leading to a 50% of maximal VEGF depletion was 104 mg/L.
Conclusion: Our results suggest that bevacizumab may be administered as 5 mg/kg weekly injections instead of every 2 weeks in patients with renal insufficiency undergoing hemodialysis. Administration may be performed anytime before or after the session on hemodialysis days.

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Measurement of tail skin temperature in rats: a model of hot flushes
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Introduction: Menopausal hot flushes, which manifest as an increase in skin temperature, occur often during the menopause. The model of disturbed tail skin temperature in ovariectomised rats is considered to be relevant to human symptoms of hot flushes.
Methods: The tail skin temperature and locomotor activity were recorded using telemetric transmitters in rats.
Results: On the first occasion, disturbed tail skin temperature was characterised in ovariectomised rats by comparison with non-ovariectomised rats: within the dark phase a maximum decrease of 6.1°C occurred in non-ovariectomised rats whereas in ovariectomised rats the decrease was only 1.9°C. No relevant difference in locomotor activity was seen.
On the second occasion, any possible effect of 17-beta oestradiol, given subcutaneously at 5 µg/kg/day was evaluated. As expected, oestradiol inhibited the elevation of tail skin temperature in ovariectomised rats with a maximum effect after 5 days of dosing. This result confirmed that the elevated tail skin temperature induced by ovariectomy is sensitive to oestrogens. After 5 days, the effect of oestradiol tended to disappear suggesting a desensitisation.
On a third occasion, raloxifen, an anti-osteoporotic drug known for inducing hot flushes was given orally at 10 mg/kg to non-ovariectomised rats. Raloxifen induced an inhibition of the decrease in tail skin temperature observed within the dark phase. This relative elevation in tail skin temperature was probably relevant to hot flushes-induced by the activity of raloxifen.
Conclusion: The model of tail skin temperature measurement in the rat can be used as a pharmacological model to evaluate possible anti-hot flush activity and also in safety pharmacology to detect possible induction of hot flushes by a test item.

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An enzyme-linked immunosorbent assay to study bevacizumab pharmacokinetics
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Introduction: Bevacizumab, a humanised monoclonal antibody (mAb) directed against vascular endothelial growth factor (VEGF), is associated to conventional chemotherapy to treat metastatic colorectal cancer. An important interindividual pharmacokinetic variability is suspected, although few data are available in colon cancer patients. An assay measuring bevacizumab serum concentrations is therefore needed to perform pharmacokinetic studies.
Methods: ELISA microtiter plates were sensitised with VEGF and saturated with phosphate-buffered saline (PBS) containing 1% bovine serum albumin (BSA). Samples diluted 1:100 in PBS-1% BSA were added and bound bevacizumab was detected using peroxidase-conjugated goat anti-human IgG specific for the Fc fragment (HRP-anti hIgG). Absorbance was measured using an ELISA plate reader. The limit of detection (LOD) was calculated by assaying 10 different drug-free serum samples and defined as the lowest concentration distinguishable from zero at two standard deviations. Each quality control sample was tested five times on one day and on six separate days. Trough and peak serum concentrations of bevacizumab were measured in 13 metastatic colon cancer patients. Data were described using a two-compartment population pharmacokinetic model.
Results: LOD was 0.033 µg/mL. The intraday precision indices of the method were (per cent coefficients of variation, CV%) 7.1%, 4.0% and 6.0% for 3.06 µg/mL, 30.9 µg/mL and 74.8 µg/mL, respectively. The corresponding bias measures (per cent deviation) were +4.1%, -5.9% and +2.1%, respectively. The between-days precision was 8.6%, 4.1% and 9.5% for 3.06 µg/mL, 30.9 µg/mL and 74.8 µg/mL, respectively. The corresponding bias were +4.1%, -5.9% and +2.1%, respectively. Lower limit of quantification (LLOQ) and upper limit of quantification (ULOQ) were therefore 3.06 µg/mL and 74.8 µg/mL, respectively. Mean (SD) trough and peak concentrations were 46.9 (27.6) µg/mL and 180.8 (57.7) µg/mL, respectively. Estimated pharmacokinetic parameters (interindividual CV) were Vc = 3.5 L (22%), CLc = 0.01 L/h (23%), Vp = 2.0 L (14%), CLp = 0.03 L/h (8%), T½-α = 0.6 days (18%) and T½-β = 21 days (16%).
Conclusion: We have developed a reproducible method for measuring serum bevacizumab concentrations in treated patients, with a standard curve ranging from 3.06 to 74.8 µg/mL. These preliminary results suggest a high interindividual pharmacokinetic variability in colon cancer patients.

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Critical evaluation of the ferret emesis model in safety and efficacy pharmacology studies
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Introduction: For pharmacological studies on emesis, the ferret is the most widely used model because of the high sensitivity of its vomiting reflex, in contrast to rodents, and because it is simpler and more economical than using larger species (e.g. dogs or pigs). Evaluation of vomiting in ferrets can be used for safety evaluation of compounds developed for anesthesia/analgesia, oncology, as well as asthma and other inflammatory diseases. In addition, it is a valuable model for evaluating efficacy against the emetic side effects of morphine-like treatments, cytotoxic agents or ionizing radiation. However, depending on the topic of the studies (safety or efficacy), the mechanism of action investigated (type of receptors) and the therapeutic class of the substance under evaluation, the experimental protocols and methodologies have to be carefully adapted.
Methods: This work describes the critical aspects of the ferret model and provides examples of results and methodologies to be considered in the context of screening, safety or efficacy investigations.
Results: The emetic effects (retches and vomits) observed with morphine following different routes of administration (p.o., i.p., or i.v.) shows the different sensitivity of ferrets to the same emetogenic agent administered via different routes. Retching and vomiting episodes induced by ascending doses of morphine (0.1–10 mg/kg i.p.) are distributed as a bell-shaped curve (55.0 ± 12.9 retches and 4.8 ± 1.4 vomits maximum at 0.3 mg/kg). Side behavioural effects are also noted at highest doses (e.g. sedation). The emetic effects obtained following repeated administration with morphine (0.4 mg/kg i.p., three test sessions with one-week or two-week washout periods) demonstrates the problems of reproducibility, which need to be considered for routine screening studies performed using the same colony of ferrets. The reduction of cisplatin-induced emesis by ondansetron (0.01–8 mg/kg p.o.), used as antiemetic agent, shows the absence of clear dose-effect relationship (no effect at 0.03 mg/kg, 100% inhibition at 0.1 mg/kg). Finally, data obtained from ferrets implanted with telemetry devices demonstrates the sensitivity of EMG recordings from the diaphragm for the investigation of the late phase of emesis in efficacy studies using the chemotherapeutic agent cisplatin (5 and 10 mg/kg i.p.).
Conclusion: These findings highlight some critical issues that need to be considered when using the ferret emesis model and demonstrate the usefulness of a sensitive EMG methodology for investigation of delayed emesis.

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Passive and active smoking: analysis of nicotine and cotinine content in hair using liquid chromatography, gas chromatography, capillary electrophoresis and colorimetry
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Introduction: Tobacco smoke is a risk factor to human health through direct consumption and/or indirect exposure via the environment. In particular, many non-smokers are passive smokers, involuntary inhaling smoke in public places or from active smokers whom they live or work with nicotine and cotinine, a primary metabolite of nicotine after C-oxidation via the cytochrome P450, have been widely used as biomarkers to determine cigarette smoking status and estimate the exposure to environmental tobacco smoke. To validate data on both passive and active tobacco exposure, we investigated the use of hair samples for quantifying nicotine and cotinine by gas chromatography, high performance liquid chromatography and capillary electrophoresis, and nicotine total metabolites by colorimetry.
Methods: Hair was taken from 15 non smokers and 15 smokers. This hair was incubated in a NaOH solution at 100°C for 1 h. The samples were then extracted using diethyl-ether. Nicotine content was evaluated using gas and liquid chromatographies and capillary electrophoresis. We used colorimetry for nicotine total metabolites levels (in cotinine-equivalents) measurements.
Results: Active cigarette smoking exacts a continuing toll on public health, it is an established risk factor for cancer and hypertension, and predisposes to atherosclerotic vascular disease. Moreover, recent evidence that inhalation of environmental tobacco smoke (i.e. Passive smoking) may also have deleterious cardiovascular effects has enormous public health implications. Passive smoking is a very common event associated with increased susceptibility to respiratory tract infections and to lung cancer. In this study, we found nicotine and cotinine in hair from smokers obviously, but also in hair from non-smokers, evincing the latter's exposure to environmental smoke. Gas chromatography showed a mean nicotine concentration of 2.13 ± 0.20 ng/mg in smokers' hair and 0.22 ± 0.08 ng/mg in non-smokers' hair. Values obtained from liquid chromatography were 1.93 ± 0.42 ng/mg and 0.51 ± 0.12 ng/mg, respectively. Capillary electrophoresis measurements indicated a mean nicotine content of 1.24 ± 0.59 ng/mg-hair for smokers and of 0.35 ± 0.11 ng/mg hair for non-smokers. We found with colorimetry an average concentration of nicotine total metabolites (in cotinine equivalents) of 19.6 ± 1.9 ng/mg-hair in smokers and of 4.45 ± 0.69 ng/mg-hair in non-smokers. Our quantification methods applied to hair samples proved reliable and provided reproducible results, allowing us to precisely assess the degree of tobacco impregnation through active and passive smoking. In all non-smoker subjects, we find non negligible nicotine levels in hair, evidence of a dangerous passive exposure to cigarette smoke.
Conclusion: Quantification experiments on hair samples reveal (reliably and reproducibly) significant levels of nicotine and its metabolites in non-smokers.

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Mycophenolic acid: utility of monitoring
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Introduction: Mycophenolic acid, the active immunosuppressant form of the prodrug mycophenolate mofetil, is a widely used component of immunosuppressive regimens in organ transplantation. Therapeutic drug monitoring is needed because of a narrow therapeutic window, an interindividual pharmacokinetic variation and a good correlation between suppressive activity and concentrations.
The aim of this study is to investigate mycophenolic acid inter individual variability of pharmacokinetic parameters and to seek for correlation between mycophenolic acid plasmatic concentration and doses administered.
Methods: It consists on a retrospective study (2001–2005). Plasmatic levels are monitored routinely by high performance chromatography. Therapeutic interval is 2.5–4.5 µg/mL.
Results: Four hundered and ninety-four samples carried from 177 subjects were evaluated. The average age was 27 years (5–63 years) with 61% men and 39% women (sex-ratio 1.58). Sixty-six per cent of patients were treated for a renal

transplantation, 20% had bone marrow transplantation, 6% had hepatic transplantation and 5% had a corticoresistent nephrotic syndrome. The average administered dose is 1527 mg/day (500–3000 mg/day). Our results show that the mean plasmatic mycophenolic acid concentration is 2.3 μg/mL (0.03–43 μg/mL), 70% of samples (137 patients) had an infratherapeutic concentration, 19% were in therapeutic interval and 11% were toxic. Statistical analysis showed no correlation between mean mycophenolic acid doses and plasmatic concentrations in all patients.
Conclusion: Our results support interindividual pharmacokinetic variability reported. Prediction of mycophenolic acid exposure based on dose alone can lead to drug plasmatic concentrations that are lower or higher than desired. The results indicate that mycophenolic acid plasmatic levels should be monitored routinely to maximize efficacy and minimize toxicity.

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Importance of hydroxychloroquine monitoring during the treatment of acute *Coxiella burnetii* endocarditis

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Introduction: Q fever endocarditis caused by *Coxiella burnetii* is rare and mainly observed in patients with immunodeficiencies or cardiac abnormalities. Evolution is lethal without therapy. The specific treatment of Q fever endocarditis is the synergic association of hydroxychloroquine and doxycycline. The range of hydroxychloroquine therapeutic levels is narrow and a drug monitoring is highly recommended. We report here a case of a *C. burnetii* endocarditis in an obese, dialysed, 68-year old woman bearing prosthetic cardiac valves, which required the determination of hydroxychloroquine plasma levels. The aim of this study is to highlight the need of a regular hydroxychloroquine therapeutic monitoring in Q fever, especially with patients who may exhibit unusual pharmacokinetics.
Methods: Positive diagnosis of *C. burnetii* endocarditis was established through echocardiography and bacteriological assay. As medical treatment did not control the cardiac failure, replacement of the affected valve became mandatory. At day 7 post surgery, a treatment with doxycycline (200 mg/day) combined with hydroxychloroquine (200 mg/day) was initiated. Hydroxychloroquine plasma levels were measured by HPLC with native fluorescence.
Results: For the first two weeks, plasma levels were low (0.2 μg/mL), showing the treatment was ineffective. Such values could result from an inadequate posology in light of the patients' obesity. Later, measurements revealed toxic levels (2.2 μg/mL), leading to a prompt administration interruption. Regardless, values remained high (2.0 μg/mL), probably because of a reduced drug elimination due to renal insufficiency. Patients with valvular damage or prosthetic heart valves are the most at risk to develop Q fever, and endocarditis is its most serious complication, leading to death without adequate antibiotic therapy. *C. burnetii* is an obligate intraleucocytic Gram-negative organism, explaining the lack of efficiency of most antibiotics. The addition of a lysosomotropic agent such as hydroxychloroquine restores the bactericidal activity of doxycycline. For endocarditis, 18 months of doxycycline supplemented with hydroxychloroquine is currently the best therapy. It shortens the treatment duration and reduces the number of relapses. In case of a Q fever, monitoring is highly recommended to reach the narrow hydroxychloroquine therapeutic range (0.8–1.2 μg/mL.). Overdosage induces serious toxic effects (e.g. Myocardial, CNS and respiratory depression, coma). Conversely, under dosage would be inefficient and risk lethal relapses. Furthermore, this weakened patient exhibited additional risk factors, namely obesity and advanced chronic renal insufficiency, thus heightening the need of monitoring.
Conclusion: This case report underlines that hydroxychloroquine monitoring is highly important to prevent overdosage toxicity or underdosage inefficiency.

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Plasmatic levels study of vancomycine

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Introduction: Monitoring vancomycin levels is highly recommended for the purpose of identifying and treating vancomycin overdose and to ensure appropriate and safe therapy. Because of its ototoxicity and nephrotoxicity vancomycin should be used with care to treat infections in patients especially with renal insufficiency. There is a little consensus about prescription of vancomycin in patients with both normal and failure renal function
The aim of our study was to evaluate prescription of vancomycin in our patients and to seek correlation between posology and plasmatic levels.
Methods: It was retrospective study (March 2001 to December 2005) about 984 plasmatic levels of vancomycin from 346 hospitalized patients. Vancomycin concentrations were carrying out by fluorescence polarization.
Results: The way of administration of vancomycin was: 85% intravenous (16% in continue, 60% discontinue and 9% non determined), 10% intraperitoneal, and 5% non determined
The usual daily administration of vancomycin in adults with normal renal function varied between 1 and 4 holds day, nevertheless patient who have nephrotoxicity get vancomycin one to three times per week (34% of patients).
Posology of vancomycine varied between 0.01 and 6 g per day with median equal to 1.5 g. The mean plasma concentration was 11.46 μg/ml (0.063–57.29 μg/ml). Results show also that 43% of patients have an infratherapeutic concentration, 21% were in therapeutic interval and 36% have a toxic concentration.
Statistic analysis shows no correlation between mean doses and vancomicyne concentration in all patients.
Conclusion: Our study confirm that optimisation of monitoring vancomycin plasma levels is recommended for adjusting dosage.

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Methotrexate falsely toxic levels

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Introduction: Methotrexate is an antifolate drug. Intravenous high-dose, administered at doses of 500 mg m^{-2} or higher, is used in several malignant diseases and requires pharmacokinetic monitoring to identify patients at risk for developing toxicity and to control folinic acid rescue, started at 12 h post-infusion. Forty-eight hour safe plasmatic levels must be less than 0.2 μmol L^{-1}.
Methods:
Case-report: We report a case of very high and persistent falsely toxic methotrexate plasmatic levels in a 12-year-old child.
Results: The patient was treated with chemotherapy including methotrexate every 70 days for an acute lymphocytic leukaemia. In the last cycle, he received 7.5 g of methotrexate over 24 h and folinic acid as each cycle and methotrexate plasmatic levels were 0.7 and 0.15 μmol L^{-1} respectively at 24 and 48 h post-infusion. There wasn't any sign of toxicity and folinic acid was stopped at 48 h. The child went home with oral rout prescribed methotrexate (30 mg/week). 10 days after infusion, he consulted the out-room department for asthenia, vomiting and mucositis. A slow redistribution of methotrexate from extra vascular tissues was suspected. Requested methotrexate plasmatic level (carried by the mother) was very high (2323 μmol L^{-1}). Because of this unusual result, the level was controlled, at first, by fluorescence polarisation immunoassay, then, by high performance liquid chromatography. The results were similar and the child was hospitalised. All drugs' intake was stopped. Hydration and urinary alkalinization started and folinic acid rescue was instituted. Two days later, even though there was no clinical sign of toxicity, plasmatic concentration was 3000 μmol L^{-1}. But, in front of the normal clinical state, the patient went home. He came back for a control one week later. Methotrexate plasmatic level was 3027 μmol L^{-1} without any clinical signs of toxicity, he went home with an appointment in 2 weeks. Inspite of clinical state normalization, follow-up showed persistent high metho-trexate plasmatic levels. Investigations on the discordance between clinical and biological data eliminated measurement errors and pharmacokinetic problems and proved a deliberate methotrexate addition in each child blood sample brought by his mother to the laboratory. So, the child's doctor carried him self the blood sample. Plasmatic level was 0. Next day, the child blood sample was brought by the patient's mother and plasmatic level was 339 μmol L^{-1}. We confirmed this hypothesis by measuring, in the same day, methotrexate plasma-tic levels in a child sample brought, by his mother and the other collected by us in our laboratory. The results were respectively: >10 000 μmol L^{-1} and equal to 0.
Conclusion: We discovered that the mother added methotrexate to the child blood samples in order to hospitalize her son.

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Determination of a limited sampling strategy for the estimation of mycophenolate AUC in renal transplant children

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Introduction: Mycophenolate has become the main antimetabolite used in the prevention of graft rejection in renal transplant children. Therapeutic drug monitoring based on the area under the curve (AUC) is recommended for mycophenolate, but full pharmacokinetic (PK) profiling is strenuous, cumbersome and costly. The aim of this study was to determine a limited sampling strategy (LSS) for the determination of mycophenolate AUC in a pediatric renal transplant population.
Methods: We conducted a retrospective study on 73 full 12 h PK profiles, collected in 50 renal transplant children between January 2005 and February 2006. Patients were 9.3 ± 4.8-year-old at time of graft and mean time from graft was 1023 ± 1226 days [0 – 4873]. Children all received an induction treatment and were on ciclosporin, mycophenolate mofetil (MMF) and corticosteroids. MMF was assayed in plasma using an immunoassay (EMIT). The best LSS was defined by multiple linear regression (MLR) analysis derived from the 73 AUC. Criteria were defined for the determination of LSS: the number of samples had to be limited to 3, samples had to include the trough and be collected within 4 h following drug intake. Validation of the best LSS was done on a separate group of 13 full PK profiles collected in 12 patients between March and May 2006. Full MMF AUC were calculated using the linear trapezoidal rule from samples drawn pre-dose (C0) and at 30 min, 1, 2, 3 and 12 h post-dose (C0.5, C1, C2, C3, C12).
Results: Mean MMF dose was 528 ± 238 mg, and mean AUC over the 12-h dosing interval calculated by the trapezoidal rule was 65.3 ± 34.8 μg mL/h (15.3–174.9). A total of six time combinations were tested. The best LSS was obtained with C0, C1, C3, and AUC equation was defined as such: AUC = 5.759 + 2.584*C0 + 1.304*C1 + 6.16*C3. Among all sampling time combinations tested, this LSS allowed the best correlation between AUC determined by MLR and AUC calculated with the trapezoidal rule (r^2 = 0.961). It exhibited the best precision (rmse = 10.74 μg/mL/h) and the lowest bias (1.76%). Mean AUC calculated by MLR was 64.1 ± 31.6 μg mL/h (19.6–155.3). The difference between AUC calculated by MLR and AUC calculated by the trapezoidal rule was not statistically significant (P = 0.47).
Conclusion: MMF exposure in pediatric renal transplant patients can be reliably predicted by a LSS including the trough and on a shorter and less strenuous sampling period than for full AUC determination. This strategy is now routinely used in kidney transplant children followed in our clinical setting. However, target MMF AUC still remain to be clearly defined.

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Assessment of Amprenavir plasma Cmin levels in patients receiving once-daily fos-amprenavir in combination with either 100 or 200 mg ritonavir

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Introduction: Although once daily (OD) administration of Fosamprenavir (fAPV) is not recommended in France, this may be done in some patients, especially when other anti-retrovirals (ARVs) are given OD. The aim of this study was to assess serum Amprenavir (APV) trough concentrations in patients given OD fAPV boosted with either 100 mg or 200 mg ritonavir (RTV).

Methods: This retrospective cohort study was conducted in all the 51 patients from a single center, who had been given 1400 mg fAPV OD between March 2005 and June 2006. Blood was sampled 24 ± 2 h after the last drug intake, within first 3 months after fAPV was started. APV and RTV trough concentrations were assessed using an HPLC method combined with a diode array. Concentrations are expressed as median (Q1–Q3). Non parametric and Chi-square tests were used as appropriate.

Results: Of the 51 patients, two patients were excluded from analysis because of non adherence to treatment. Median APV Cmin were 1.52 ng/mL (IQR 1.13–1.90) and 1.34 ng/mL (IQR 0.91–1.66), in patients given 200 mg (n = 24) and 100 mg (n = 25) RTV, respectively. APV Cmin was <0.375 μg/mL in one patient from each sub-group. RTV Cmin were below quantification limit in 13/24 patients receiving 200 mg RTV and in 18/25 patients receiving 100 mg RTV (P = 0.009). By month 6, no virologic failure was observed among the 13 PI-naïve patients and among the 13 patients who had been switched from BID to OD fAPV/RTV. Conversely, they where 4 virologic failures among the 15 patients who had a prior history of failure to a PI-containing regimen.

Conclusion: APV Cmin were within expected range in most patients and were not statistically different whether RTV dosage was 100 or 200 mg. This correlated with high virologic success rates in PI-naïve patients and in patients switched from BID to OD fAPV.

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Contribution of CYP 3A4 and CYP 2D6 in loratadine metabolism *in vitro*

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Introduction: Loratadine (LOR) is a long acting tricyclic antihistamine with selective peripheral histamine H_1-receptor antagonist activity. The Cytochromes P450 (CYP) 3A4 and 2D6 have been previously described as contributors to LOR metabolism. The aim of our study was to compare the respective contribution of CYP 3A4 and 2D6, that are still controversial in loratadine metabolism.

Methods: Loratadine was incubated with recombinant CYP isoforms as well as pooled human liver microsomes, in the presence or absence of chemical inhibitors. LOR and desloratadine (DES) determination was performed by LC-MS/MS.

Kinetic analysis was performed both by measuring the disappearance rate of the parent compound (LOR) and the production rate of its major metabolite (DES).

Results: Among the 9 isoforms tested only CYP 1A2, 2B6, 2D6, 2C19, 3A5 and 3A4 were involved in LOR metabolism, with CYP 3A4, CYP 2D6 being the most active isoforms. CYP3A4 exhibited a higher metabolic activity for both the formation of DES (Cl_{int} = 12.25 vs. 5 μL/min/mg) and LOR disappearance (Cl_{int} = 135.7 vs. 15.45 μL/min/mg). These findings were supported by the chemical inhibition studies in pooled human liver microsomes: the CYP 3A4 inhibitor ketoconazole (2 μM) strongly inhibited LOR disappearance and DES formation, by 84.3% and 66.4% respectively. In contrast, quinidine (10 μM), a CYP2D6 inhibitor induced weaker inhibition (16.5% and 33.0%, for LOR disappearance and DES formation, respectively).

Conclusion: Taken together, these results provide evidence that CYP2D6 and mainly CYP3A4 are the major isoforms responsible for the metabolism of LOR.

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Monitoring of ganciclovir concentrations in renal transplant children

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Introduction: Cytomegalovirus (CMV) infection is a frequent complication of solid organ transplantation, usually transmitted by the donor organ, and is responsible for increased morbidity and mortality in transplanted children. CMV infection can be treated by ganciclovir (GCV), a synthetic guanine derivate that inhibits replication of herpes viruses including CMV. GCV is available as an intravenous formulation. When administered orally, its bioavailability is limited to 5–10%. For that reason, GCV can be administered as its prodrug, valganciclovir. CMV resistance can be induced by sub-therapeutic GCV concentrations. To minimize resistance, through (C0) concentration should always be over 0.5 mg/L. The aim of the present study was to describe GCV monitoring and pharmacokinetics in renal transplant children, and to determine if they had the recommended GCV blood levels.

Methods: This study is a retrospective analysis of GCV monitoring performed in 36 kidney transplant children treated for a CMV infection between June 1999 and August 2006. Patients were aged 10.0 ± 4.9-year-old at time of graft. Antiviral treatment consisted of either IV or oral GCV, or oral valganciclovir. Monitoring of GCV was conducted prospectively and based on C0 or on area under the concentration-time curve over the twelve-hour dosing interval (AUC). Dosing adaptation was based on the recommended minimum C0 concentration. GCV plasma concentrations were measured by HPLC with UV detection. 74 full GCV AUC were calculated using the linear trapezoidal rule from samples drawn pre-dose (C0) and 1, 2, 4, 8 and 12 h post-dose (C1, C2, C4, C8, C12).

Results: 23/35 children received a kidney from a donor whose status was CMV+, and 12 of them (34%) were CMV-. Mean time between graft and treatment initiation was 89 ± 111 days, and mean course duration was 120 ± 77 days. Mean doses were 5.9 ± 1.6 mg/kg for IV GCV, 49.2 ± 10.0 mg/kg for oral GCV and 15.6 ± 5.5 mg/kg for oral valganciclovir. After IV GCV administration, mean C0 was 0.91 ± 1.19 mg/L (n = 47) and mean AUC was 35.3 ± 17.3 h.mg/L (n = 29). After oral GCV and oral valganciclovir administration, mean C0 were respectively 1.00 ± 0.84 mg/L (n = 94) and 1.51 ± 1.84 mg/L (n = 71), and mean AUC were respectively 16.9 ± 9.0 h.mg/L (n = 18) and 47.5 ± 14.3 h.mg/L (n = 27). C0 and AUC were statistically different between the 3 treatment groups (P < 0.05). Overall, C0 was over 0.5 mg/L in 69% cases (50% for IV GCV, 66% for oral GCV and 86% for oral valganciclovir).

Conclusion: This study allowed a description of GCV PK in renal transplant children treated for CMV infection. It showed a statistically significant difference of exposure to GCV depending on the type of drug used (IV GCV vs. Oral GCV vs. Oral valganciclovir). According to the recommendations, children had a suboptimal GCV plasma concentration in almost 30% cases. As PK of GCV is highly variable, monitoring GCV C0 is recommended to avoid CMV resistance.

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Documentation of Voriconazole plasma levels in immunocompromised pediatric patients

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Introduction: Voriconazole (VRC), antifungal triazole agent, is effective against a broad spectrum of pathogens, including *Aspergillus*, *Cryptococcus* and *Candida* species. VRC exhibits a linear pharmacokinetics in children; but elimination capacity is in part correlated with CYP2C19 genotype. Little is known about the plasma pharmacokinetics in immunocompromised children at risk for invasive fungal infections (IFI).

Methods: We retrospectively analysed pediatric patients receiving VRC for 'proven', 'probable' and 'possible' IFI (EORTC-BAMSG) during 2006. A simple specific HPLC method was used for determination of VRC and its hydroxymetabolite (OH-VRC) in children plasma.

Results: Data from a total of 19 plasma samples from seven caucasian children, aged 2–17 years old (mean age: 11) were analysed. Mean weight was 28 kg [13–66]. Four patients have received haematopoietic stem cells transplant, two suffered from leukemia, and one from lymphoma. In three patients Aspergillosis infection was documented, in two, suspected and the two others were treated for a possible Aspergillosis. Median VRC dose was 8 mg/kg/day for a median duration of 53 days. Median VRC trough levels was 0.9 mg/L (0.1–3) and median OH-VRC trough levels was 2.4 mg/L [1.6–4.6]. OH-VRC levels were always superior to VRC level. Mean metabolisation ratio was 2.6 [1.3–4.7].

Among the three patients with aspergillosis infection, two had a complete response associated with a VRC trough levels >1 mg/L. The third patient died. His VRC trough level was <1 mg/L in spite of VRC dose increase. In the other cases, all responded to VRC treatment, with trough levels >1 mg/L in two cases.

Conclusion: In the literature, a relationship between efficacy and voriconazole plasma levels was observed in adults (P < 0.025) (1). Our results, despite the small samples size of this study, suggest a relationship in pediatric patients. To our knowledge, no data are available concerning OH-VRC trough levels. Large inter-individual variations in VRC and OH-VRC levels renforces the useful of therapeutic drug monitoring in optimizing VRC efficacy.

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Lopinavir population pharmacokinetics on HIV-infected adults – body weight and co-treatments influence

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Introduction: Lopinavir is a protease inhibitor (PI) used in HIV-infection treatment with a current recommended dosing regimen of 400 mg (with 100 mg ritonavir) BID. The aim of our study was to develop a population pharmacokinetic model highlighting the possible influence of co-variates such as bodyweight and other anti-retroviral treatments. Such a model could be useful to evaluate the current recommended dose with regard to lopinavir target plasma concentrations.

Methods: The pharmacokinetics of lopinavir were investigated using a population approach performed with NONMEM on 709 HIV-1-infected patients (1275 samples). Individual Bayesian estimates of lopinavir pharmacockinetic parameters were used to calculate the minimal concentrations obtained with various dosage regimens. The probability to achieve previously determined target concentrations in PI-naïve (i.e. 3 mg/L) and PI-pretreated (4.7 and 6 mg/L) patients was calculated for each regimen. Influence of bodyweight on the probability to achieve these target concentrations was evaluated by logistic regression.

Results: Lopinavir pharmacokinetics was well described by a one-compartment model, with typical population estimates (interindividual variability %) of 4.5 L/h (34.1%) and 33.5 L (43.5%) for apparent clearance and distribution volume, respectively. Bodyweight, concomitant treatments with nevirapine, efavirenz, and amprenavir were found to have an influence on lopinavir pharmacokinetics. The 400 mg BID regimen enables 94% of the population to reach a minimal concentration 12 h after last intake of 3 mg/L, but enables only 79% and 45% of the population to reach a 4 and 5.7 mg/L concentration. Furthermore, a 10 kg increase in bodyweight significantly decreased the probability to reach target concentrations for PI-pretreated patients when using a 400 mg BID regimen (odds ratios of 0.57 and 0.56 with P < 0.0001).

Conclusion: The 400 mg lopinavir-100 mg ritonavir BID regimen is efficient to reach a minimal concentration of 3 mg/L for PI-naïve patient but may not be suitable for PI pretreated patients. Bodyweight has an influence on the minimal 12 h after intake concentration and should be taken into account when initiating a therapy to a pretreated patient.

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Ribavirin plasma concentrations and virological response to peginterferon plus ribavirin therapy in HCV-HIV coinfected patients previously treated with standard interferon combined or not with ribavirin

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Introduction: In HIV-infected patients, treatment of hepatitis C virus (HCV) infection is associated with poor virological responses. The influence of ribavirin (RBV) concentration on the sustained virological response (SVR) to RBV-based regimen in HCV-HIV co-infected patients is evaluated.

Methods: HCV-HIV co-infected patients were included in an observational, open-labeled and controlled trial. They received RBV between 800 and 1200 mg/day in combination with 180 µg of pegylated interferon-α2a weekly for 48 weeks. Sustained virological response (SVR) was defined as an undetectable HCV RNA 24 weeks after the end of the treatment. Blood samples were collected at weeks 2, 12, 24. RBV was quantitated in plasma using a HPLC method and UV detection between 1 and 12 h postdose. HCV genotypes, CD4 count and HIV RNA levels were assessed. An univariate analysis was conducted between ribavirin (RBV) plasma C_{min} concentration and virological results to determine RBV cut off.

Results: Seventeen patients were enrolled in the study. Medians of demographic variables were 40 years-old, 72 kg, 1.01 mg/dL for serum creatinine and 10.88 L/h for estimated creatinine clearance. 9 (60%) patients were HCV genotype 1 infected. SVR occurred in 14 patients (46%). RBV concentrations were purchased from 84 blood samples. The most discriminating statistic value for determination of RBV C_{min} efficacy-threshold was estimated to be 1.9 µg/mL at W24.

Conclusion: The results suggest that RBV dosage, initially based on weight and HCV genotype, should be henceforth optimized to maintain RBV plasma C_{min} above 1.9 µg/mL in HCV-HIV co-infected patients.

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Population pharmacokinetics of ropivacaine after local peri-urethral anaesthesia for incontinence surgery

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Introduction: Urinary incontinence surgery is often performed under local anaesthesia using peri-urethral infiltration. We occasionally observed high ropivacaine plasma concentrations during such surgery which may lead to cardiovascular or neurotoxicity. Since ropivacaine kinetic studies during this kind of surgery are lacking, the present study aimed to described ropivacaine pharmacokinetics with this unusual route of administration.

Methods: Twelve female patients (mean age: 58 years, from 24 to 84 years) suffering from urinary incontinence were included, after obtained a written consent. They received 3 peri-urethral infiltrations with 15 mL of ropivacaine 7.5 mg/mL (total dose 112.5 mg). Five blood samples were retreived after the 3rd infiltration until 8 h post-dose. Times off sampling were different between the patients (sparse data). Total and free ropivacaine determinations were measured by gas chromatography with nitrogen-phosphorus detection. Potential symptoms of toxicity were collected. A population pharmacokinetic analysis was performed to estimate the parameters using the NONMEM software.

Results: A 2-compartment model best described the data with a zero-order input and a first-order output. Inter-individual variability on clearance and the central volume of distribution were defined as proportional. The residual variability was described as additive. A first order with conditional estimation (FOCE) method was used throughout. The following pharmacokinetic parameters were estimated: central clearance (0.23 L/min), the central volume of distribution (21 L), the intercompartimental clearance (7 L/min), the peripheral volume of distribution (19.1 L) and the virtual duration of the infusion (2.43 min). The inter-individual variability of clearance and central volume of distribution were 55 and 110%, respectively. The residual variability was 0.73 mg/L. The standard error of all pharmacokinetic parameter estimations was below 27%. One patient developed an acute neurotoxicity during the infiltration (paresthesia, hot flushes).

Conclusion: The data showed that plasma levels observed after peri-urethral infiltration for urinary surgery are above threshold concentration for CNS toxicity. This route of administration leads to a faster and a more extensive blood diffusion compared to other peripheral blocks. Cautions should be taken by anaesthesist when using a peri-urethral infiltration.

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Voriconazole monitoring in cystic fibrosis patients post lung transplantation

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Introduction: Voriconazole has become a preferred agent for the treatment of aspergillosis. It is a broad-spectrum antifungal triazole with non-linear pharmacokinetics secondary to saturable clearance. The pharmacokinetic (PK) properties of this drug have been described before, mostly in healthy volunteers. Relationship between drug concentration level and positive clinical response is not clearly established in ill patient. The aim of this study is to evaluate voriconazole plasma concentration obtained in cystic fibrosis patients after transplantation.

Methods: On this preliminary study, we retrospectively evaluated voriconazole monitoring in 14 cystic fibrosis patients (18–45-year old) post lung transplantation (immunosuppressive treatment: ciclosporin, tacrolimus or sirolimus and mycophenolate mofetil). Indications of treatment were prophylaxis, pulmonary aspergillosis, aspergilloma. Voriconazole doses were administered orally 200 mg twice daily for eight patients, 150 mg for three patients and 100 mg for three others, in function of weight. Trough voriconazole plasma levels (n = 41) were assayed using a validated high-performance liquid chromatography assay with UV detection (LOQ: 0.2 mg/L).

Results: Before dose adjustment, voriconazole mean plasma concentration was 0.85 mg/L (<0.2–2.7). 68% of the measurement were lower than 1 mg/L, 93% lower than 2 mg/L. After administration of a same dose of 400 mg (2 x 200), plasma concentration results were also from: <0.2–2.7 mg/L. Among the 14 patients, four doses were increased as a result of level: one has presented elevation of hepatic enzyme witch need drug discontinuation; for others increase of dose from 300 to 600 mg, lead to plasma concentration of 2.3 mg/L; 200–400 mg: 0.9 mg/L; 300–550 mg: 1.4 mg/L. These results illustrate an important interindividual variability in voriconazole kinetic.

J. Smith and Al in 2006 observed, in a small cohort of patients (n = 28), favourable responses in patients (n = 10) with concentrations above 2.05 mg/L, while disease progressed in of patients (n = 18) with concentrations below 2.05 mg/L.

This concentration of 2 mg/L is very difficult to reach in cystic fibrosis patients post transplant because of particular PK in this population, interindividual variability and interactions. Increase of dose expose patients to potential severe adverse effects (hepatotoxicity, neurologic effects…). Moreover 2 mg/L may not be, in this population, a predictable level of efficacy.

Conclusion: Administration of an usual voriconazole dose in cystic fibrosis patients lead to low plasma concentration, no direct link was observed between dose and plasma concentration. Others studies will be necessary to determine voriconazole pharmacokinetic factors in these patients.

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Investigation of the intestinal absorption of immunosuppressive drugs using the human intestinal Caco-2 cell line

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Introduction: The small intestine expresses a number of ATP binding cassette (ABC) transporters which could influence the absorption of commonly used oral immunosupressive drugs (IS) and play a role in their pharmacokinetic variability. The aim of this study was to investigate the transport mechanism of five IS: mycophenolic acid (MPA), calcineurine inhibitors (Ciclosporin, CsA and Tacrolimus, Tacro), and mTOR inhibitors (Sirolimus, Siro and Everolimus, Evero) using an *in vitro* model of human intestine.

Methods: Transport studies were performed using monolayers of the human intestinal epithelial cancer cell line Caco-2, cultured during 21 days on a permeable matrigel treated membrane until confluence. Permeability studies were conducted in Hank's balance salt solution (pH = 7.0) at 37°C. The integrity of Caco-2 monolayer was evaluated by measure of the transepithelial electrical resistance. Cells were incubated with 1–100 µM MPA, 0.02–50 µM CsA, 0.5–100 µM Tacro and mTOR inhibitors. IS determination was performed using validated LC-MS/MS methods. The apparent permeability coefficient (Papp) was determined in the apical-to-basolateral (a–b) and the basolateral-to-apical (b–a) directions.

Results: All IS displayed polarized transport. At the lowest concentration tested, the Papp (b–a) was 40- to 70-fold higher than the Papp (a–b) for calcineurin and mTOR inhibitors, and only 2-fold higher for MPA. IS Papp (a–b) increased whereas Papp (b–a) decreased with increasing concentrations, suggesting saturation of apical efflux transporter(s), except for Tacro whose Papp (b–a) increased with concentration suggesting the saturation of basal efflux transporter.

Conclusion: These results indicate that the intestinal transport of IS is polarized and suggest the existence of an efflux mechanism which involve saturable energy-dependent transporters. Identification of these transporters is ongoing in our laboratory.

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A well tolerated 5-FU-based treatment in a DPD-deficient patient with history of severe toxicity following capecitabine administration

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Introduction: Thirty per cent of patients receiving 5-fluorouracil as continuous infusion suffer from grade 3–4 toxicity. Some of these cases are due to a dihydropyrimidine dehydrogenase (DPD) deficiency and patients known to be deficient are usually selected for alternative treatment including non-fluoropyrimidine compounds. We report safe use of continuous infusions of 5-fluorouracil in a patient with a IVS14+1G>A associated-DPD-deficiency.

Methods: A 34-year-old woman suffering from an infiltrating carcinoma of the left breast with disseminated hepatic nodules had received successive cycles of chemotherapy including FEC100, hormonotherapy and capecitabine monotherapy (1250 mg/m^2, two times per day for 14 days). Faced to a severe capecitabine-induced toxicity, we conducted genotypic and phenotypic (dihydrouracil/uracil ratio) analyses which showed DPD deficiency related to a heterozygote IVS14+1-G > A mutation.These findings have led to preclude 5-fluorouracil use for the ongoing chemotherapy. However, considering the potency of fluoropyrimidines and ineffectiveness of subsequent chemotherapies in this patient, it was decided to administer 5-fluorouracil again, through 5-days continuous infusion with pharmacokinetic monitoring. The dose of the first course was initially set at 35% of the theoretical dose (TD) of 600 mg/m^2/d. 5-fluorouracil concentrations were monitored each morning to obtain total area under the curve (AUC) for the course. Dose increases were planned if post-course tolerance was satisfactory and if AUC was below the target of 50 000 µg/L/h.

Results: The dose had been gradually increased for each subsequent course from 75% of the TD at the 2nd course to 100% for the last two courses. The patient had fever and aphthae for 3 days and a grade 2 neutropenia (PNN = 929/mm^3) during the 2nd course but this toxicity was not considered limitative.

Conclusion: In this case report, 5-fluorouracil has been administered without major toxicity to a DPD-deficient patient, carrying the splice site mutation IVS14+1G > A, who had previously developed a severe toxicity after treatment with capecitabine. This observation indicates that tailoring 5-fluorouracil dosage in DPD deficient patients is feasible using pharmacokinetic monitoring. This case illustrates that selection of alternative treatment modalities may not remain the unique choice in these patients.

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Urinary leukotriene E4 excretion: biomarker of active inflammatory bowel disease

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Introduction: Crohn's disease (CD) and ulcerative colitis (UC) are chronic inflammatory disorders collectively referred to inflammatory bowel diseases (IBD). Leukotrienes are proinflammatory 5-lipoxygenase-derived products which play a major role in the immune and inflammatory responses because of their chemotactic and vascular effects. Consequently, the may be involved in the pathogenesis of IBD. The aims of this study were to evaluate (1) the urinary excretion of leukotriene E_4 (LTE_4) in patients with CD, UC and healthy volunteers, and (2) the relation between LTE_4 production and the activity/relapse of the disease.

Methods: We prospectively studied 34 patients with CD, 24 patients with UR and 31 sex- and age- matched control subjects. Activity of the disease was determined on inclusion by Crohn's disease activity index for CD and clinical index activity for UC. The urinary excretion of LTE^4 was measured by liquid chromatography tandem mass spectrometry. LTE_4 data are expressed as pg/mg creatinine and presented as media (10th–90th percentiles).

Results: The urinary excretion of LTE_4 was increased ($P = 0.0016$) in patients with CD: 54 (25–159) and UR: 64 (26–186) compared to healthy subjects: 33 (21–82). LTE_4 levels were significantly ($P = 0.01$) higher in patients with active disease than in patients with relapse for whom the levels of LTE^4 were similar to the level of healthy subjects.

Conclusion: 5-lipoxygenase pathway activation could contribute to the inflammation associated with IBD. The quantification of urinary LTE^4 could be an interesting non invasive biomarker for the assessment of the biological activity of CD and UC.

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A new analytical method to adapt tacrolimus posology in renal transplant patients

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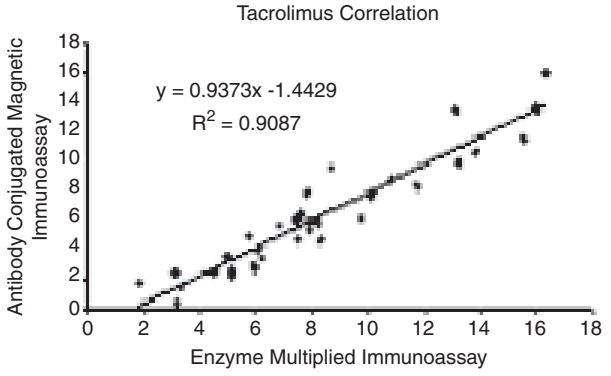
Introduction: Tacrolimus is a potent immunosuppressor drug with a narrow therapeutic index used in renal transplant patients among whom there is a significant correlation between effectiveness/side effects and residual blood concentrations. Several protocols are currently under development to evaluate the correlation between effectiveness/side effects and the area under the curve of the blood concentrations, determined with only three samples (protocol Apomygre, Opera). This correlation is important because it allows patients to increase/decrease their oral dose. However, to adapt the posology, the analysis method needs to be sensitive, specific, exact, precise and particularly rapid. The aim was to validate a new fully automated method (the Antibody Conjugated Magnetic Immunoassay) for whole blood tacrolimus monitoring and to compare with the Enzyme Multiplied Immunoassay method usually used in medical laboratories.

Methods: This validation firstly assessed the accuracy and the precision of the quality controls for both repeatability and reproducibility and secondly compared the values obtained between the two methods for the same patient samples ($n = 35$).



Results: The accuracy and the precision were lower than 15% and 10%, respectively. The correlation for patient samples is shown in Figure 1.

Figure 1 Correlation between blood concentrations of tacrolimus. (Antibody Conjugated Magnetic Immunoassay method vs. Enzyme Multiplied Immunoassay method)

Conclusion: A high correlation exists between tacrolimus levels measured by the two methods. The Antibody Conjugated Magnetic Immunoassay method may represent a reliable and valid analytical alternative given its cost effectiveness and the absence of handling errors. Thus there is a strong case for adopting it for the routine determination of blood tacrolimus concentrations in renal transplant patients.

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In the post-APOMYGRE era: ISBA, a free website for Bayesian dose adjustment of immunosuppressive drugs

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Introduction: In April 2005, we launched a free website for the Bayesian dose adjustment of mycophenolate mofetil (MMF) for both kidney and liver transplant recipients, followed by another one for ciclosporin in April 2006. The Bayesian estimators used have been published over the last 5 years in the international literature and successfully used in a mycophenolate TDM-validation trial and in a ciclosporin concentration-controlled study, respectively.

Methods: Bayesian estimation of MMF AUC0-12 h (or AUC0-8 h in case of fractionated dosing) is performed using three plasma concentrations measured at approximately 20, 60 and 180 min post-dose. Based on the estimated AUC and current MMF dose, two new doses (rounded to the nearest dosage unit) are proposed to reach the AUC0-12 h therapeutic range boundaries, i.e. 30–60 mg/h/L.

Results: For MMF, more than 2500 requests have been sent in 20 months by 20 French and 3 Belgian transplantation centres, with an average of 270 requests per month over the last 3 months. At any post transplant period, the range of AUC/dose was more than 1 to 10, with 47 to 54% patients outside the accepted therapeutic range before dose adjustment. A majority of patients were underdosed during the early post-transplantation periods, while a more symmetrical distribution between underdosed and overdosed patients was found later on. When patients were seen again after a first dose adjustment and the dose given was (still) in the previously proposed dose range (i.e. 2/3 of cases), 80–90% AUC values fell within the AUC target range at any post-transplantation period.

Conclusion: The ISBA website has been extensively used by the French and Belgian renal transplantation centres in 2006. Inter-patient variability in MMF exposure was very high and dose-adjustment was actually needed in approximately 50% of cases. Collaborative pharmacokinetic studies are going-on to develop Bayesian estimators in lung and heart transplant, as well as in lupus patients.

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Sensitive simultaneous measurement of droperidol and ondansetron used in preventing PONV with a LC/ESI/MS/MS method. Pharmacokinetic interactions study

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Introduction: Many patients suffer from postoperative nausea and vomiting (PONV) after general anaesthesia with volatile anaesthesics or propofol. Receptor that can cause nausea or vomiting or both include dopamine type 2, serotonin type 3, histamine type 1, and muscarinic cholinergic type 1 receptors. Two effective agents are droperidol, a dopamine D2 receptor antagonist, and ondansetron, a serotonin type 3 receptor antagonist. Patient at moderate risk for PONV benefit generally from the administration of one of this agent. In high-risk patients, combining the two antiemetics with different mechanism of action has been shown to be more effective than using only one agent alone. However, no data are available on pharmacokinetics of these two drugs when used simultaneously. The purpose of this study was to develop a LC/ESI/MS/MS method allowing the simultaneous determination of the two drugs in order to evaluate the potential pharmacokinetic interactions.

Methods: A new method based upon LC coupled to ion trap mass spectrometry detection with electrospray ionization interface has been developed for the identification and quantification of droperidol and ondansetron in blood. The two drugs were isolated from blood using a basic L/L extraction with ether/heptane (90/10, v/v) using haloperidol and tropisetron as internal standards. Solutes are separated on a 3-µm C18 High purity (ThermoHypersil) column (150 x 2.0 mm, ID) using acetonitrile/2 mM NH4COOH pH 3.8 buffer (40/60 v/v) as the mobile phase with a flow-rate of 200 µL/min. Data were collected either in full-scan MS mode at m/z 100–450 or in full-scan MS/MS mode, selecting the ion m/z 294 for ondansetron, m/z 285.2 for tropisetron, m/z 380 for droperidol and m/z 376 for haloperidol. The most intense daughter ion of ondansetron (m/z 212) and droperidol (m/z 194) were used for quantification. In order to study the pharmacokinetics parameters, 18 subjects received successively either droperidol (1.0 mg by intravenous route) alone, ondansetron (4 mg by intravenous route) alone, both drugs, or placebo. Blood samples were collected at the end of perfusion and 2, 4, 6, 10, 20, 30, 45, 60, 120, 240, 420 and 600 min after.

Results: Retention times were 2.63 min for ondansetron, 2.50 for tropisetron, 3.17 for droperidol and 4.77 for haloperidol. Calibration curves were linear for both compounds in the 0.50–500 ng/mL range. The limits of detection and quantification were 0.10 ng/mL and 0.50 ng/mL, respectively. The intra- and inter-assay precisions evaluated at 3, 30 and 300 ng/mL were all <6.4% and the intra- and inter-assay accuracies were in the 97.6–101.9% range either at 3, 30 or 300 ng/mL. No significant pharmacokinetic interactions between droperidol and ondansetron were observed, either for Cmax, AUC or T1/2 when each drug was used alone or simultaneously.

Conclusion: This method enables the unambiguous identification and quantification of ondansetron and droperidol. It is very convenient for both TDM or toxicological purposes. No pharmacokinetic interaction appeared between ondansetron and droperidol in healthy subjects.

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High consumption of topical non-steroidal anti-inflammatory in an osteoarthritis population

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Introduction: To describe anti-inflammatory drug use in patients suffering from osteoarthritis (OA) identified using prescription database in two French regions [Midi-Pyrénées (MP) and Provence-Alpes-Côte d'Azur-Corse (PACA)].

Methods: From the health fund's reimbursement database, we selected OA patients receiving drugs traditionally used in the management of non specific OA (chondroitin, oxaceprol and diacerhein) during the last trimester 2004. A random sample of this population matched on age, gender and geographic location on a population of Parkinson's disease patients was used for the analysis. We describe here the drugs for musculoskeletal systemused during the year 2005.

Results: The study included 4062 OA patients in MP and 7267 in PACA. Half of them were men (46.5 in MP and 44.8 in PACA). Patients in MP were younger than in PACA with a mean age of 76.5 and 77.1 respectively. Concerning geographic location, 36% lived in rural zone in MP and 10% in PACA. More of one third suffered from a chronic disease according to the definition of the health insurance system with 33.1% in MP and 37.6% in PACA. Chondroitin was most frequently used (62% in MP and 67% in PACA), followed by diacerhein (30% in the two areas) and none received oxaceprol. Concerning consumption of other musculoskeletal drug, systemic non-steroidal anti-inflammatory drugs (NSAIDs) were used by 52% of OA patients. Piroxicam was most frequently used (14%), followed by ibuprofen (12%), diclofenac (10%) and ketoprofen (9%). Celecoxib was less used (5% of OA

patients in MP and 6% in PACA). Topical NSAIDs were used by almost half of OA patients (42% in MP and 46% in PACA). Diclofenac and ketoprofen were the most represented topical drugs (20% and 16% respectively). Topical NSAIDs were more frequently used in older patients (from 36% in patients <70 years to 48% in patients >90 years in MP for example).
Conclusion: Because OA is especially common in the elderly, the chance of polymedication and adverse drug reactions heavily influence medication selection. Even if it is known that NSAIDs are able to induce cutaneous adverse drug reactions by topical application, their prescription appears relatively high in this population.

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Drug perception by children: a survey of 138 children at school
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Introduction: French people are considered as heavy drug consumers. One can hypothesize that children knowledge and perception of the drug could influence their drug consumption later.
The aim of the present study was to investigate drug consumption, knowledge and perception by children going to school in Toulouse and its metropolitan area.
Methods: 138 children (5, 9 and 13 years old) were questioned by the mean of a questionnaire.
Results: 30–40% of the children have taken a drug the day before or the day of the survey, mainly for an oro-pharynx disease. 33%, 49% and 85% of the children respectively said that they 'usually take the drug themselves'. Whatever the age, drugs containing acetaminophen, ibuprofen and aspirin were the most well-known drugs. The more appreciated pharmaceutical form was syrup and pill also for the eldest. Young children did not like injections when 13 years old children hated rectal forms. Very few children had an idea of the composition and mechanism of action of the drug: 'fighting against microbes' was the main cited mechanism. 80% of the children thought that it is useless to take a drug when you are nervous but only 58% of the eldest had the same opinion. According to the age, 40–55% of the children thought that a drug is necessary for sleep disorders. For more than 90% of the 5 year old children, a drug cannot be responsible for an adverse effect. Conversely, 58% and 79% of the children aged of 9 and 13 years respectively said that an adverse drug reaction is possible. More than 80% of the eldest thought that a drug can induce dependence.
Conclusion: Children hardly know drugs. Discussing drugs at school as soon as possible could improve knowledge and promote rational drug use.

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Pharmacoepidemiology of over-the-counter drug abuse and dependence: a pilot study
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Introduction: Over-the-counter (OTC) medicines are used to treat minor pathologies which can easily be diagnosed by the patient himself. These drugs are available without prescription and some of them contain psycho-active substances. The aim of this pilot study was to develop a pharmacoepidermiological method to investigate abuse and dependence in the context of over-the-counter medicines. This method is based on the participation of community pharmacies to collect patient data. We present here the method and the preliminary results of this pilot study.
Methods: The recruitment of pharmacies has been done from a community pharmacy network in French Midi-Pyrénées area and by pharmacy students performing a training course in a pharmacy. Volunteer pharmacies were randomly divided into five groups. We asked each pharmacy in each group to include patients requesting one drug from the list of available over-the-counter drugs corresponding to the four following substances: codeine, dextromethorphan, pseudoephedrine and H1 antihistamines. The control group includes patients requesting anti-acid drugs. Patients were asked to fill in an anonymous questionnaire investigating patterns of drug use and criteria of abuse and dependence according to the Diagnostic and Statistical Manual IV (DSM-IV) definitions. The questionnaire can be filled inside or outside the pharmacy. The acceptability of the questionnaire has been tested over a two-week period.
Results: Calculation of the daily dose and comparison to the maximal dose recommended will characterise abuse. Dependence or abuse is considered when three of the Diagnostic and Statistical Manual IV criteria are met. To validate the method, we expect a difference in the number of cases of abuse and/or dependence between the control group and the other ones. Pharmacies and patients recruitment rates, answer rates according to the location of questionnaire completion will allow evaluation of the study's feasibility. The association between abuse and dependence and other variables will also be investigated: age, gender, socioprofessional categories, psychotropic drugs consumed. Finally, potentially addictive medicines should be identified.
Conclusion: The results of this pilot study should identify methodological issues in conducting surveys in pharmacies and underline the key role of pharmacists in the evaluation of drug abuse and dependence. This approach could complete knowledge about psychotropic drugs in the specific context of over-the-counter medicines.

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High dosage buprenorphine consumption after ten years of marketing, data from oppidum program in 2005
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Introduction: In France, two maintenance programmes for opiate users are available: methadone and high dosage buprenorphine (BHD). BHD is now used since ten years and some misuse was reported. The aim of this study was to evaluate the consumption of BHD in 2005 and to analyse a sub-group of consumption of BHD without protocol.
Methods: The Oppidum Program (Observation of illegal drugs and misuse of psychotropic medications), a multicentric survey, annually surveys drug dependent subjects attending specialised care centres throughout France. Data were collected by questionnaire on sociodemographic variables, and drug use during the preceding week.
Results: During October 2005, 74% ($n = 2695$) of subjects were under maintenance programmes for opiate users. In the high-dosage buprenorphine group ($n = 1311$), 94% ($n = 1233$) subjects were identified as 'within protocol' (consuming the drug in a strict care protocol, with a correct follow-up) and 6% ($n = 78$) 'outwith protocol' (consuming the drug without any supervision). Many differences were noted between the two groups. For the subjects (within protocol) ($n = 1233$), the daily dose is 8.3 per mg. 90% of subjects used orale route, 13% intravenous route and 8% the nasal route. 11% of subjects report cocaine drug use, 13% heroin and 23% benzodiazepines. The 'outwith protocol' subjects ($n = 78$) displayed more misuse behaviour. They reported more illicit drug use (heroin: 37% and cocaine: 22%), more frequently used an intravenous route (34%) or nasal route (30%), Buprenorphine is procured illegally in 86% of cases.
Conclusion: These results underline, once again, the potential misuse of BHD: others routes used (intravenous or nasal) witch expose to somatic complications, drugs associations, deal.

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Utilization of analgesics in medicine and biology: an ISI database approach (2003–2005)
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Introduction: The aim of this study is to quantify utilization of a prescribed analgesic: codeine, and two over-the-counter analgesics: ibuprofen, and acetaminophen, in the scientific fields of Medicine and Biology.
Methods: Using the *ISI Current Contents Life Sciences* and *Clinical Medicine* databases for the 2003–2005 period, publications including the terms 'codeine' or 'ibuprofen' or 'acetaminophen' or 'paracetamol' in their title, abstract or keywords, were retrieved; and several bibliometric indicators (author country affiliation, impact factor -IF of the journal, …) were computed.
Results: Concerning the dataset analysed, scientific papers involving acetaminophen (1626 publications) dominated, followed by papers involving ibuprofen (910) and papers implicating codeine (300), most of them being published in the *Life Sciences*. The most prolific countries were the USA, and the United Kingdom; and were followed by several industrialized countries (Germany, France, Japan, Italy, Canada…); China, India, and Turkey were ranked between 10th and 15th. For all drugs, the scientific literature production of the European Union challenges that of the USA.
Codeine: papers were published in 156 journals, the most prolific being *Forensic Science International* (IF: 1.388, 20 publications); appearing mainly in journals belonging to *Chemistry & Analysis* subdiscipline of *Life Sciences*, or journals belonging to the *Research/Laboratory Medicine & Medical Technique* subdiscipline of *Clinical Medicine*.
Ibuprofen: papers were published in 388 journals, the most prolific being *The International Journal of Pharmaceutics* (IF: 2.039, 31 publications); appearing mainly in journals belonging to *Pharmacology & Toxicology* subdiscipline of *Life Sciences*, or journals belonging to *Pharmacology & Toxicology* subdiscipline of *Clinical Medicine*.
Acetaminophen: papers were published in 539 journals, the most prolific being *The International Journal of Pharmaceutics* (41 publications); appearing mainly in journals pertaining to *Pharmacology & Toxicology* subdiscipline of *Life Sciences*, or journals pertaining to the *Anesthesia & Intensive Care* subdiscipline of *Clinical Medicine*.
Conclusion: The predominance of acetaminophen in the scientific literature regarding the utilization of other analgesics (ibuprofen, codeine) is noted. In addition, the contrast between papers involving codeine (often published in journals whose scope is related either to toxicology or to forensic sciences), and papers involving ibuprofen or acetaminophen (appearing mainly in the fields of pharmacology or pharmaceutics) is also noted.

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Treatment in diabetic patients – pharmacoepidemiological research into the consumption of antidiabetic drugs
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Introduction: To determine the profile and therapeutic management of diabetic patients using drug consumption data in two French regions [Midi-Pyrénées (MP) and Provence-Alpes-Côte d'Azur-Corse (PACA)].
Methods: The population source was defined as being all people covered by the public health insurance system (80% of the general population). All patients having received any diabetic treatment, including insulin and oral hypoglycaemic drugs (OHAs), during the last trimester 2004 were identified from the health insurance database. A random sample of this population matched on age, gender and geographic location to a population of Parkinson's disease patients was used for the analysis. We analysed all drugs and care reimbursed in 2005.
Results: Four thousand, one hundred and sixty-two diabetics in MP and 7297 in PACA were studied. Approximately half of them were men (46.8 in MP and 45.0 in PACA) with a mean age of 77 ± 10 years (SD). Concerning therapeutic regimen, thirteen per cent of the patients were treated only with insulin (16.4% in MP and 9.4% in PACA), 70% only with OHAs (67.3% in MP and 75.8% in PACA) and 13% with the association of insulin + OHAs (14.0% in MP and 12.7% in PACA). There was a significant increase in insulin prescription in MP (30.4%) over that prescribed in PACA (22.1%; $P < 0.001$). By contrast, OHAs were more frequently used in PACA (88.5%) than in MP (81.3%; $P < 0.001$). Among OHAs, the sulphonylureas and metformin were the most commonly used (60.3% and 45.8% respectively), followed by the α-glucosidase inhibitor (10.5% in MP and 13.9% in PACA), repaglinide (10.8% in MP and 9.1% in PACA) and finally thiazolidinediones (4.5% in MP and 6.2% in PACA). Over the age of 80, 76.4% of patients were prescribed OHAs in MP and 86.8% in PACA. Concerning the consumption of cardiovascular drugs, hypolipidaemic drugs were consumed by half of the diabetics with 72% receiving statins and 28% fibrates. ACE inhibitors were used by one third of the patients (33.5% in MP and 32.7% in PACA). Concerning the medical care of those patients, only one third had a consultation with an ophthalmologist, 12% with a diabetologist and 13% with a dentist during the year 2005.
Conclusion: In 2005, the patterns of antidiabetic drug use were different in the two regions. Most of the patients are still treated with OHAs even when there are over 80 years of age. Most of the diabetic patients were managed by general practitioners and were not referred to a specialist. The results suggest that the guidelines are not being followed particularly for the annual screening for diabetic retinopathy.

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Data collection agreement in pharmacoepidemiology: comparison of patients, physician and database sources

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Introduction: Measure of drug exposure is essential in pharmaco-epidemiology. It can vary depending on the source that is used. It is sometimes assessed using patient and/or physician interviews, but also health care claims data. In this study, we aimed to compare data collected from three sources: the patient, the physician and the French National Health Insurance System for salaried persons, (CNAM-TS) database.

Methods: Between the 1 of August 2003 and the 31 July 2004, patients who received at least one dispensation of NSAID were randomly sampled monthly from the CNAM-TS. Patients and prescribers were asked to fill in a questionnaire regarding sociodemographic characteristics, NSAID indication and use, and previous medical history. Healthcare resources used in the 6 months preceding inclusion were extracted from the French national healthcare insurance database. Were compared: medical histories and NSAID indication as reported by the patients and the physicians; type of prescription (initiation or renewal of treatment) as indicated in the three sources; concomitant gastroprotection separately between patient or physician report and the database. The agreement between the sources was measured using the kappa statistic (K). Concordance was considered poor for $K < 0.4$, medium between $K \geq 0.4$ and <0.6 and good for $K \geq 0.6$.

Results: A total of 26 618 subjects for which data were available from the three sources were considered for analysis. A good concordance was observed between patient and physician for cardiovascular disorders (e.g. arterial hypertension: $K = 0.77$), NSAID indication (e.g. inflammatory rheumatism: $K = 0.71$) and between physician and database for concomitant gastroprotection: kappa = 0.63. Concordance was medium for report of hypercholesterolemia ($K = 0.53$, patient vs. Physician), for type of prescription ($K = 0.53$ patient vs. Physician, $K = 0.52$ physician vs. The database). Between patient and physician, concordance was poor for gastrointestinal history ($K = 0.39$) and for osteoarticular indications ($K = 0.22$). Concordance between patient report and the database for gastroprotection was borderline ($K = 0.40$).

Conclusion: Data information varies between sources. Discrepancies may be due to the absence of knowledge of certain previous medical histories. It could also be attributed to the subjectivity of symptoms (e.g. digestive disorders). Poor agreement between patient and database for gastroprotection could be due to self-medication by the patient.

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Medicinal plants and treatment of diabetes in Senegal: survey with patients

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Introduction: Diabetes is a major public health problem. Its frequency increases every day in all countries. However, in developing countries like in Africa, few people have access to medicinal drugs. In addition, in Africa, traditional believes induce people to use medicinal plants for the treatment of their health problems. Thus, many people in these developing countries use plants for the treatment of their diabetes. However, few work focused on the knowledge and attitudes of users on medicinal plants in Africa in general and in Senegal in particular. That's why we undertook this work with the general aim to evaluate the use of medicinal plants for the treatment of diabetes in Senegal in order to make recommendations for their best use or their surrender.

Methods: This study was done in a reference centre for the management of diabetes in Dakar. The questions were in general closed and focused on the name of the most used plants, the reasons of using plants, the suppliers of plants, the perception of patients on the efficacy of medicinal plants, the doses used and the adverse effects of plants. We did a cross- sectional survey by direct interview. A representative sample of 220 patients was interviewed from May 2 to June 30, 2006. The results are processed by Epi Info and given as percentage.

Results: Forty-one plants were cited by the patients and the 5 most frequently cited were *Moringa oleifera* (65.90%), *Sclerocarya birrea* (43.20%), *Alium sativum* (6.4%), *Terminalia avicennioides* (5.5%) and *Qarcinia cola* (5%). Patients declared several reasons for using medicinal plants (traditional treatment: 40%, efficacy: 32%, low cost: 20%). The principal suppliers of plants were sellers in the market (66.8%), traditional therapists (5%) and structures of traditional medicine (3%). Sixty-five percentage of patients think that medicinal plants are efficient for the treatment of diabetes and 35% consider that medicinal plants are not efficient. More than half of patients (55.5%) said that they have been informed on the doses to use and 44.5% have any indication on the doses. Forty-four patients (20%) had adverse effects which could be caused by medicinal plants. These adverse effects were in general gastric disorders and two cases of hypoglycaemia and one coma were also noted.

Conclusion: Many people in our study think that medicinal plants are efficient for the treatment of diabetes and, that's why, scientists in developing countries must work in order to proof the efficacy and the innocuousness of herbal medicine.

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Non rational use of high dosage buprenorphine: Comparison of use's pattern between 2000 and 2005

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Introduction: Many studies have reported High Dosage Buprenorphine (HDB) misuse. 'Non rational' users have been associated with an increased misuse of HDB or benzodiazepines. In France, ease of access to HDB has contributed to diversion to the illegal market through doctor shopping. Recommendations of good clinical practices and Health System regulatory interventions were stated in 2003/2004. The aim of this study was to assess if those interventions modified the proportion of non rational users in Haute-Garonne in ambulatory care.

Methods: This observational cohort study included new HDB users identified from the French Health System reimbursement database in Haute-Garonne between January 2005 and October 2005. All drug deliveries were followed for 6 months. Patients were considered as rational, non rational or occasional users according to the number of HDB prescribers and HDB community pharmacists consulted. We also studied concomitant use of at least one reimbursement of benzodiazepines during the follow up. We compared these patients to those included in a previous cohort with the same design in 2000.

Results: Four hundred and twenty-seven new HDB users were included in 2005 (mean age 34.1 ± 8 year, 72.8% men, mean number of HDB reimbursements 9.3 ± 8.4 (1–64)). Among them, 48.7% were ?rational' (≤2 prescribers and ≤2 pharmacies), 23.8% 'non rational' (>2 prescribers and/or >2 pharmacies), and 27.5% 'occasional' (respectively 42.6%; 28.7%; and 28.7% in 2000, $n = 242$). There was no significant decrease in the proportion of 'non rational' users within 2000 and 2005 studies. Characteristics of 'rational' users remain stable between 2000 and 2005. Among 'non rational' users, HDB daily delivered quantities have not decreased in 2005 (14.8 ± 23.2 vs. 13.3 ± 18.1 mg in 2000, ns). The proportion of 'non rational' users receiving more than 16 mg per day increased significantly (28.9% vs. 23.1%, $P < 0.001$), as well as the proportion of doctor shopping (85.6% vs. 71.6%, $P < 0.001$). If the use of some benzodiazepines decreased (flunitrazepam, dipotassium clorazepate), 'non rational' users were still more exposed than 'rational' users to clonazepam, bromazepam and dipotassium clorazepate in 2005. Clonazepam daily delivered quantities were superior in 'non rational' than in 'rational' users in 2005 (median 8 vs. 3 mg).

Conclusion: In France, regulatory measures on HDB have been partially assessed. This study shows that medical and pharmacies' shopping remains a significant problem among new users. Moreover, there is no improvement in the behaviour of the most 'deviant' new HDB users. Generalisation of clonazepam use with indicators of misuse is alarming.

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Severe drug-induced hyperkalemia: a retrospective study in Midi-Pyrenees

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Introduction: Hyperkalemia may lead to serious and even fatal cardiac complications. Besides several risk factors, many drugs can cause or aggravate hyperkalemia especially potassium sparing diuretics (such as spironolactone). Moreover, since spironolactone is widely prescribed for cardiac insufficiency in association with other drugs known to induce increase in potassium plasma levels, hyperkalemia seems to be a frequent adverse drug reaction in clinical practice. This study was designed to assess the responsibility of drug intake in severe hyperkalemia.

Methods: The study involved all hospitals or clinics including a nephrology unit in Midi-Pyrenees area ($n = 11$). Subjects were selected using computerized databases of biology laboratories according to the following criteria: (i) age of at least 18 years, (ii) hospitalization in nephrology, cardiology, geriatrics, emergency or intensive care units, (iii) dosage of plasma potassium concentration higher or equal to 6.5 mmol/L between 1st January and 31st December 2005. All patients on chronic hemodialysis for terminal renal insufficiency were excluded. Medical records were consulted to collect data about patients (socio-demographic characteristics, medical history), hyperkalemia (circumstances of onset, risk factors, clinical signs, treatment) and drug use.

Results: Preliminary results concerning five centres are shown. Among 136 observations of severe hyperkalemia collected, 68 (50.0%) could been caused or aggravated by the use of at least one drug. 35 subjects (51.5%) were men and mean age was 77 years. 89.7% of patients suffered from cardiovascular risk factors or diseases (mainly hypertension or cardiac failure). Potassium plasma values ranged between 6.5 and 8.8 mmol/L (mean value: 7.0 mmol/L). In most of the cases (95.6%), hyperkalemia was associated with one or more of the following risk factors: acute and/or chronic renal insufficiency, diabetes, dehydration, metabolic acidosis. Hemodialysis was used to treat hyperkalemia for only 10 patients (14.7%). 50.0% of subjects used spironolactone when hyperkalemia occurred and in 66.7% of these subjects, spironolactone was associated to angiotensin converting enzyme inhibitors or angiotensin II receptor antagonists. A potassium supplements intake was also found in 23.5% of cases.

Conclusion: The use of laboratory databases allows an exhaustive selection of cases of hyperkalemia. Even if inclusion criteria of this study were relatively strong (hospitalized patients, high limit of potassium plasma concentration), the present results show a high number of drug-related severe hyperkalemia probably due to a lack of surveillance of ambulatory patients treated with drugs known to raise potassium plasma level.

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Transmission of adverse drug reaction information in discharge summaries: an observational study

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Introduction: Prevention of recurrence of adverse drug reactions is based on identification, management and information of health care professionals in charge of the patient, in particular the general practitioner. This is the first evaluation of the medical follow-up of patients suffering from adverse drug reaction along their stay at the hospital and of transmission of adverse drug reaction information in discharge summaries.

Methods: A 1 month prospective observational study on patients admitted in the emergency unit and suffering from adverse drug reaction was conducted. Patients included were followed-up during all their hospital stay. Chart review in emergency unit and in the different downstream units, if applicable, was performed paying special attention on discharge summaries. Comparison of data regarding adverse drug reaction was done at all the different stages.

Results: On the 3967 patients admitted during the period, 135 presented 138 adverse drug reactions: incidence rate 3.4%. Adverse drug reactions are of A type in 97%, serious in 82%, avoidable in 20%. Adverse drug reaction was the reason for admission in 72.5%. 41% of the patients went out after the consult, and 59% are admitted to downstream units. Spontaneous notification rate is 2.2%. Regarding management of adverse drug reaction: emergency units modified treatment in 25.4%, in 42% management is delegated to downstream unit or to specialist practitioners, 11.6% of treatments are modified before entry. In the downstream units: 18.5% results in posology modification, 81.5% drug switch or withdrawal. Regarding transmission of information: 52% of adverse drug reaction are mentioned in the final discharge summary, for those expressly diagnosed, up to 80% are mentioned. In case of direct exit to home, 43% are mentioned in emergency discharge summary, 24% in a explicit way. Between emergency unit and downstream units, there is a loss of 29.6%. Within the downstream units, when mentioned at arrival, 81.5% of ADR are mentioned in discharge summaries, 33.5% in a explicit way.
Conclusion: Computerised chart existence since several years and participation to such evaluations show engagement of emergency units in quality enhancement. Bias possibilities exist: lack of sensitivity of chart review because of oral transmission of information to patients and physicians, because of inhomogenous chart filling and discharge summaries writing within the different hospital units. But these findings strongly invite to promote good chart filling and to systematically include an 'adverse drug reaction' item both in medical chart and in discharge summary. Exhaustive filling will be time costly, but shall be considered as a useful investment to avoid recurrences and subsequent additional costs.

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Zolpidem abuse: regional health insurance database analysis

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Introduction: Zolpidem, available in France since 1987, is an hypnotic medicine which therapeutic treatment is limited now to one 10 mg pill a day. The aim of this study is to evaluate the use and the misuse of zolpidem in Southern France.
Methods: Analysis of data from 'Bouches-du-Rhône' department health insurance database for 2003. A consultation with a medical practitioner of the regional health insurance system was proposed to the treated patients using the value or more than the threshold of three pills a day in order to improve the knowledge of the zolpidem consumption. The data were collected on paper forms and included in an EXCEL software with an anonymized analysis.
Results: One hundred and twenty-five patients ingest an average value of three pills a day or more corresponding to 0,16% of the population with a prescription of zolpidem during 2003. The mean quantities of pills during 24 h is from 3 to 79. The average number of medical doctors consulted for prescription is 8.3 (from 1 to 75 for men and 1 to 56 for women). The average number of pharmacies where zolpidem was delivered was 9.6 (from 1 to 80 for men and from 1 to 77 for women). 91 of the 125 patients were seen by the insurance medical practitioner. 70 of them declare to try to decrease or to stop zolpidem with or without medical assistance. 36 patients declare to have withdrawal symptoms including seven cases of seizures. In 2005 41% of the same patients use less than three pills a day and 11.4% of them totally stopped.
Conclusion: This study emphasize the interest of the health insurance database in the evaluation of drug dependence, use and misuse of medicines. The obtained results confirm the supposed data of misused and/or dependence zolpidem. The consultation with the insurance medical practitioner seems to have a positive impact on abuses.

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Treatment in Parkinson's disease – Pharmacoepidemiological research into the consumption of Antiparkinsonian drugs

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Introduction: The aim of this study was to describe antiparkinsonian drug use in patients with Parkinson's disease (PD) identified using health insurance system databases in two regions in France [Midi-Pyrénées (MP) and Provence-Alpes-Côte d'Azur-Corse (PACA)].
Methods: We selected all patients receiving antiparkinsonian or anticholinergic drugs during 1st October to 31 December 2004. Antiparkinsonian drugs were levodopa, dopamine agonists (bromocriptine, Pergolide, Ropinirole, pramipexole, lisuride), anticholinergics (Biperidene, trihexyfenidyl, tropatepine), selegiline and entacapone. Patients younger than 35 years old and receiving only anticholinergic and neuroleptic drugs were excluded. Drug reimbursements were collected for the year 2005.
Results: The study identified 4162 PD patients in MP and 7304 in PACA. About half of them were men and their mean age was 77 ± 11 years with a range of 35–103. Concerning geographic location, 32% lived in rural zone in MP and 10% in PACA. At least, 75% of PD patients suffered from a chronic disease according to the definition of the health insurance system. Levodopa was most frequently used (82% in MP and 85% in PACA), followed by dopamine agonists (31% in MP and 35% in PACA) and anticholinergic agents (10% in MP and 7% in PACA). Selegiline and entacapone were prescribed in 8% of PD patients in the two regions. Finally, amantadine was less used (only in 5% of PD patients). About 55% of PD patients received drug in monotherapy (85% levodopa alone and 6% dopamine agonists), 25% of patients received two drugs, 10% three drugs and 5.5% four or more. Levodopa was more frequently used in older patients (from 65% in patients <70 years to 90% in patients >90 years) whereas dopamine agonists were frequently used in younger patients (from 56% in patients <70 years to 15% in >90 years). These patterns of antiparkinsonian drugs use were similar in the two regions.
Conclusion: This study shows that the therapeutic scheme in Parkinson's disease in two areas in France appear similar and closely related to guidelines for treatment. Using a large and accurate database, it is possible to describe the pharmacological treatment of Parkinson's disease.

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Validity of benzodiazepine exposure measured from patient interview: data from the 3C cohort

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Introduction: In pharmacoepidemiology, measure of drug exposure may vary depending on the source used. Often assessed using patient and/or physician interviews, many studies also use health care claims data. In this study, we aimed to validate benzodiazepine exposure as measured using patient interview and taking health care claims data as a reference.
Methods: The 3C study is a prospective cohort that included from March 1999 to March 2001, 9294 community-dwelling elderly persons of 65 years old or more living in Bordeaux, Dijon or Montpellier. Drug exposure was collected from subjects by face-to-face interwiews at inclusion, 2 years and 4 years after their inclusion. Claims data from the CNAM-TS were available from the second interview for 3136 of the included subjects. From the date obtained by interviews, we classified as exposed to benzodiazepine, subjects who report taking benzodiazepine at both the two consecutive follow-up exams. From the claims data, we classified as exposed to benzodiazepines, subjects having at least 80% of expected claims between the two follow-up interviews. We considered two different maximal delays between claims: 60 days and 120 days. Using claims classification as a reference, we estimated validity (sensitivity, specificity, positive and negative predictive values) of the interviews.
Results: According to the interviews, 598 subjects were considered as exposed to benzodiazepines. According to the claims data and a maximal delay between claims of 60 days, 142 subjects were classified as exposed which corresponded to an exposure prevalence of 4.5%. Sensitivity and specificity were 83.0% and 84.0% respectively. Positive and negative predictive values were 19.7% and 99.1% respectively. Using a maximal delay of 120 days, 247 subjects were classified as exposed which corresponded to an exposure prevalence of 19.7%. With this less conservative definition, sensitivity, specificity and negative predictive values did not vary greatly (86.2%, 86.7%, 98.7% respectively), whereas, due to elevated prevalence, positive predictive values increased to 35.6%.
Conclusion: This study demonstrates a certain discrepancy between measuring exposure with interviews and health care claims data which can lead to misclassification bias.

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A French consensus panel for determining potentially inappropriate medication use in the elderly

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Introduction: In order to assess drug-related problems in the elderly, several lists of potentially inappropriate medications have been proposed by Beers (US) and Mac Loed (Canada), using an expert consensus panel. These lists are not however adapted to French practice. In France, the list adapted for the 3C cohort had some limits: lack of a consensus method, exclusion of criteria (dose, medical condition), availability of drugs. The objective was to establish a list of inappropriate medications for the French elderly using the Delphi method.
Methods: The 2-round-Delphi method was used; the aim was to collect responses of experts from a first round and then to ask these same experts to complete a revised questionnaire based on the results obtained from round 1. The goal of the Delphimethod is to converge to an agreement from the average response of the participants. The study was organized in four phases: (i) creation of the preliminary questionnaire of inappropriate medications from a literature review, (ii) recruitment of 15 experts (6 pharmacologists, 5 geriatrics, 2 pharmacists, and 2 general practitioners with clinical geriatric qualification from various locations in France), (iii) mailing of the round 1 questionnaire, (iv) mailing of the round 2 questionnaire based on round 1 synthesis.
Results: The final list contained 34 criteria: 29 medications or medication-classes applied to all patients aged 75 years and over, and five criteria with medications that should be avoided in specific medical conditions. Among these 34 criteria, 25 were considered with an unfavourable benefit/risk ratio, one criterion with questionable efficacy and eight criteria with both unfavourable benefit/risk ratio and questionable efficacy. Six criteria were not judged by experts as inappropriate: dextropropoxyphene-paracetamol, fluoxetine, amiodarone, long-term prescription of NSAIDs, and long-term prescription of NSAIDs for patients with a history of hypertension or renal failure.
Conclusion: We propose a list of explicit criteria for determining potentially inappropriate medication use in the elderly (75 years and over) in France, based on an expert consensus. This consensus grouped practitioners commonly involved in the management of drugs given to elderly people which allowed a large overview of medical practice in France. As drug-related problems are a public health priority, this tool could help minimizing drug-induced accidents and additional costs.

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Does the end of refund of phytotherapy involve a defer towards other refunded specialities?

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Introduction: The reimbursement of phytotherapy were reconsidered recently by the transparency committee on 2005. Because of methodological weakness in the demonstration of their effectiveness, the end of refund for these drugs was decided on 1 March 2006. Nevertheless, the transparency committee has noted that the specialities of phytotherapy may limit the recourse to psychotropic drugs such as benzodiazepines and the hypnotic ones in the minor forms of the concerned disorders. Thus, it appeared interesting to follow up the possible defer towards these types of specialities, after the end of refund of the drugs of phytotherapy.
Methods: From the database of regional health insurance system concerning the Franche-Comte area, we compared delivered volumes of drugs by classes EMPHRA for a 6 months period before (October 2005 to March 2006) and after (April to September 2006) the end of refund of the twelve specialities classed as 'hypnotic or sedative drugs of phytotherapy'.

Results: A total of 811 000 'social policy-holders' was recorded in the regional database from which 33 315 'social policy-holders' have received at least once hypnotic or sedative drugs of phytotherapy in the previous 6 months before the end of their refund.
The evolution of the total deliveries in volume between the two periods for the two populations are as follows: - 13% for the whole population and – 26% for the population previously consuming phytotherapy.
Specifically, the amount of deliveries of psychotropic drugs (benzodiazepines and other type of hypnotic and sedative drugs) was dropping twice more quickly among the population previously consuming phytotherapy than in the whole population, between the two periods of observation.
We are going on with this survey for the next 6 months, in order to prevent a seasonal effect and we are performing the follow-up for sales of the drugs of phytotherapy before and after the end of their refund from the wholesaler in pharmacy.
Conclusion: These results seem to contradict the assumption that consumption of drugs of phytotherapy would have referred on other refundable specialities.

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A modelled French population to assess the baseline cardiovascular risk
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Introduction: The population impact assessment of preventive strategies requires knowledge of the baseline characteristics which determine the risk of the event to be prevented. Such information is not easily available because of the lack of epidemiological representative studies, or differences among methods employed in data collection or recordings.
Our aim was to construct a simulated population appearing like the French population, enabling to explore the impact of various preventive strategies of cardiovascular events.
Methods: We obtained real data from national statistics and the Monica – France cohort. In order to reproduce the risk structure of the French population, we considered (i) demographic statistics; (ii) risk factors distribution and (iii) equations linking the risk factors to the incidence of cardiovascular events.
By using probabilistic algorithms we re-created virtual but realistic individuals taking into account the individual-specific features such as the vector of subject demographics and covariates as age, blood pressure, together with the covariance between these characteristics. We tested the validity of the constructed population by comparison to the original variables distributions.
We employed SCORE and Framingham equations to assess cardiovascular baseline risk of each virtual subject. Endpoints were (i) total cardiovascular mortality; (ii) coronary heart disease mortality; (iii) cardiovascular non coronary mortality. We compared the prediction to the observed incidence of events in the French population.
Results: The simulated population was similar to the original cohort, as shown by their covariates distributions.
Regression analysis between the predicted risk in the simulated population and the real incidence in French population showed an overestimation of risk for all endpoints with the exception of the non coronary death in men with Framingham equations. Risk prediction by SCORE method was adequate in both sexes.
Conclusion: We propose an original approach enabling to create a realistic virtual population from different sources of data. This reproducible method can find many applications, such as offering a reference to the individual prediction of risk, the prediction of public health impact of therapeutic strategies, or comparing the performance of risk prediction scores.

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Prevalence estimation of adverse drug reactions by a three sources capture-recapture method
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Introduction: The aim of this study is to provide an estimation of prevalence of adverse drug reactions by a three sources capture-recapture method and an estimation of exhaustivity of these three sources. This method allow to estimate the number of cases which are not identified by any source, the number of total cases, and by the way, exhaustivity of each source.
Methods: The source population is inpatients who went out from the Grenoble teaching hospital in February 2006. The study population was these inpatients presenting an adverse drug reaction identified in one at least of the three sources during this period: local pharmacovigilance register of spontaneous reporting, discharge summaries from the GESTDIAG® software (developed for the medical information unit for coding purposes) and data extracted from the 'programme de médicalisation des systèmes d'information' using the International Diseases Classification (10th version) codes suggesting adverse drug reactions. Identification of common case was made by comparing each source to the two others. The software used to do the statistical analysis with log linear regression models was STATA®.
Results: 245 cases of adverse drug reaction were identified by reading of discharge summaries. 57 cases were identified after extraction from the 'programme de médicalisation des systèmes d'information'. 19 cases were spontaneously reported. One case was common to the three sources. The best model was the independant model (without interaction between the three sources), for which confidence interval was the narrowest, Akaike Information Criterion was the lowest (−3.3.7) and Bayesian Information Criterion was the lowest too (−9.02). The number of lacking cases was 913 (397–1429), very near of the number obtained with the weighted Bayesian Information Criterion (900). The number of total cases was 1218 (702–1734). During this period, 9489 inpatients were registred. So prevalence of adverse drug reactions during February is 12.8% (7.4–18.4). Exhaustivity of discharge summaries is 20.1% (14.1–34.9), of the 'programme de médicalisation des systèmes d'information' is 4.7% (3.3–8.1) and spontaneous reporting is 1.5% (1.0–2.6).
Conclusion: A sensitivity analysis should have been done to see if the number of common cases was enough to give reliable results. But as the prevalence results were very near of these obtained by several other pharmacoepidemiologic studies, it has not been conducted. Spontaneous reporting rate is low compared to previous studies. Discharge summaries appear to be a promosing tool for adverse drug reaction detection and survey.

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Assessing general practitioner's prescribing behaviour in elderly patients with concealed renal failure
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Introduction: In elderly patients serum creatinine level may be normal despite decreased glomerular filtration rate (GFR). Because many drugs are excreted through the kidney, the risk of adverse drug reaction is increased if the dosage is not adjusted to renal function. The necessity of dosage adjustment is probably underestimated in clinical practice and no study had considered this issue in general practice. The objective of this study was to assess general practitioner's (GP) prescribing behaviour in elderly patients with concealed renal failure and their knowledge of drugs for which a dosage adjustment is necessary.
Methods: The study was carried out in a French department. GPs were randomly select to achieve a questionnaire including clinical cases simulating drug prescription in elderly patients with concealed renal failure and two questions exploring the concern of GPs relating to the evaluation of GFR in elderly patients. The questionnaire was answered with the interviewer and the use of the RCP (2003 edition of the Vidal dictionary) was allowed.
Results: Fifty GPs agreed to participate in study. Before prescribing to an elderly patient (>70 year-old), 28% of GPs evaluate the GFR for all new drug, 32% only for certain drugs and 51% regularly even for a renewal of drug. 80% of the GPs tell take into account the creatinine clearance for the prescription of drugs in elderly patients. But for the prescription, 52% of GPs consider renal function to be normal on the only value of the serum creatinine level (while it was decreased) and only 14% of them really calculate clearance with the Cockroft and Gault equation. Thirteen GPs (26%) consulted the Vidal dictionary at least once before answering. For the prescription to a 70-year-old patient whose clearance was estimated at 50 mL/min, GPs did not decrease, despite a need for that, the dosage of allopurinol (54%), sotalol (64%), digoxine (44%) and levofloxacine (48%). For the prescription to a 75-year-old patient with severe renal failure (clearance at 25 mL/min), GPs prescribe dextropropoxyphen (86%), celecoxib (38%) and nadroparine at therapeutic dose (74%), despite a contra-indication.
Conclusion: GPs are not enough sensitized about the reduction of GFR in elderly patient and probably underestimate the risk related to decreased drug elimination in mild renal impairment. A part of them do not know the limits of GFR evaluation by serum creatinine level and do not systematically use the clearance estimated by Cockroft and Gault. Main drugs which are contra-indicated or whose dosage must be adapted to renal function require to be better known.

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Comparison of abuse potential of psychotropic medications in real life settings
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Introduction: Doctor shopping is one of the principal mean of diversion for psychotropic medications. Abuse potential of psychotropic medications in real life setting can be approached by measuring doctor shopping on prescription databases. We performed an analysis of doctor shopping in a French region to assess the abuse potential of the five major psychotropic medications classes.
Methods: We extracted all drug deliveries reimbursed by the General Health Fund during year 2005 in PACA-Corse region (population 4.93 millions) for oral route formulations of five classes of psychotropic medications (benzodiazepines, antidepressants, opiate maintenance therapy, stimulants and neuroleptics). We used two indicators to evaluate the abuse potential of each class: doctor shopping ratio (percentage of total delivered quantity delivered obtained by doctor shopping) and corrected doctor shopping quantities (number of DDD obtained by doctor shopping among each class, discounting a basic level of doctor shopping of 0.5%) given in thousands of DDD (kDDD). The same indicators were also given for the compound with the most important doctor shopping ratio within each class.
Results: Doctor shopping ratios were 12.1% for opiate maintenance therapy (corrected doctor shopping quantity: 441 kDDD), 3.03% for benzodiazepines (2 941 kDDD), 2.15% for stimulants (4 kDDD), 1.04% for neuroleptics (103 kDDD), 0.79% for antidepressants (212 kDDD). The compounds with the most important doctor shopping ratios were: buprenorphine for opiate maintenance therapy (14,4%, 402 kDDD), flunitrazepam for benzodiazepines (30.3%, 429 kDDD), methylphenidate for stimulants (2.4%, 3 kDDD), cyamemazine for neuroleptics (1.4%, 45 kDDD) and tianeptine for antidepressants (2.0%, 73 kDDD).
Conclusion: Abuse potential is highest for opiate maintenance therapies and benzodiazepines. A significant abuse potential for stimulants is noted despite restrictive prescription and delivery conditions in France. Except for the particular case of tianeptine, no significant abuse potential was detected for antidepressants.

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Adherence to asthma controller medication: Evidence from the French health insurance data
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Introduction: Although guidelines and progress to optimize asthma treatment have been made, many studies have suggested that asthma control in the general population is inadequate. The aim of this study was to assess adherence to asthma anti-inflammatory controller medication (AAICM).
Methods: Patients aged from 15 to 45 years with one or more record for AAICM in the social security insurance database of Aquitaine, from the 1st of December 2003 to the 31st of December 2003 were included. The date of first reimbursed record was defined as the index date. Patients were grouped according to their AAICM: (i) Inhaled corticosteroid alone (ICS), (ii) ICS in association with long-acting inhaled ß2-agonist (LABA) in a single inhaler (ASSO), (iii) ICS in association with LABA in separated inhalers (DISSO), (iv) Antileukotrienes (ALT). Patients included in the DISSO group had renewal prescriptions of ICS and LABA within an

interval of 30 days. For each patient, we collected sociodemographic data and all drug-reimbursement records between the 1st of June 2003 and the 28th of May 2005. New users were defined as patients with no reimbursement for any asthma controller medication in the previous 6 months before the index date. Adherence was assessed by (i) medication availability (using the 'Continuous Multiple-interval measures of medication Availability' (CMA) definition), which gives information on the coverage of a treatment, and (ii) persistence in time. CMA below 80% was defined as poor adherence. Treatment persistence was estimated measuring the duration over which patients have not stopped their treatment by Kaplan–Meier survival curves analysis. Discontinuation was defined as a maximum gap of 30 days between the theoretical end date of prescription and the start date of the following one. For groups treated with ICS (alone, combined or dissociated), discontinuation was considered if patient stopped the ICS. All patients were followed for 17 months.

Results: A total of 12 502 subjects were identified. The mean age was 32 years (SD = 8.9) and 41.7% were male. 43% were new users.

CMA was below 80% for 85%, 79%, 62% and 63% of new users of ICS, ASSO, DISSO and ALT respectively, and for 70%, 46%, 24.5%, 26% of previous users. Persistence rates after 17 months were 20%, 31%, 78%, 38% for new users of ICS, ASSO, DISSO, ALT and 28%, 40%, 68%, 69% for previous users.

Conclusion: Adherence to anti-inflammatory treatment is weak in asthma in new users. Special attention should be paid to patients treated by ICS alone and to new users where the adherence rate is the lowest.

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Abuse and misuse indicators of benzodiazepines and benzodiazepines-like drugs: Data from OPPIDUM survey in 2005

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Introduction: The aim of this study is to assess abuse and diversion indicators of use/misuse of benzodiazepines and benzodiazepines-like drugs using OPPIDUM program (Observation of Illegal Drugs and Misuse of Psychotropic Medications) performed in October 2005.

Methods: OPPIDUM is a annual, national and multicentric survey describing consumption profile of psychoactive substances. CEIPs (Centers of Evaluation and Information on Pharmacodependance) organize the data processing and their collection from drug addict patients met in health care centers.

Consumption characteristics of BZD have been analysed and particularly misuse indicators (search of positive effects, concomitant intake of alcohol, illegal acquisition…).

Results: In the survey of 2005, 23% of patients used BZD. The more used were bromazepam (127 times reported), clonazepam (115), diazepam (111), alprazolam (111), oxazepam (108), diazepam (98), zolpidem (76) and flunitrazepam (59). Flunitrazepam remain the leader for a lot of misuse indicators (77% of dose superior to recommended dose, 82% of abuse/dependence, 89% of positive effects, 56% of illegal acquisition). Clonazepam indicators are also high.

Conclusion: Consumption characteristics of flunitrazepam and clonazepam are remarkably different of other BZD, underlying a higher misuse for this two drugs. If the number of observations related to flunitrazepam has decreased since several years, the increase of clonazepam should be follow closely in the next surveys.

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Evaluation of risk of thromboembolic event associated with exposure of combined estrogen and progestin or progestin-only of women from poitou-charentes area: a case-control study

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Introduction: Data from clinical trials and observational studies showed that combined oral contraceptives and hormone replacement therapy increased risk of thrombo-embolic event. Data were collected from western or north European countries for most studies. Women concerned by these results were not representative of French population and combined estro-progestin used in these studies were not frequently used in France. A similar risk with progestin-only exposure was not well documented. The aim of this study was to investigate thromboembolic risk of combined estrogen and progestin or progestin-only in a French population.

Methods: A population-based case-control study was conducted between March 2004 and June 2006 among Poitou-Charentes resident women aged 18–65 year. Investigators were hospital and non-hospital practitioners. Cases were women who presented an incident venous thrombosis or a pulmonary embolism or a cerebral thrombosis. Controls were women unaffected by a thromboembolic event and were matched with the corresponding case by 5-year age band and area of residence. Pregnancy or malignancies were exclusion criteria. Information on exposure and on acquired/inherited risk factors were collected in a face-to-face questionnaire and a blood sample was drawn to evaluate biological status.

Results: One hundred and forty-four cases and 281 controls have been included in this study. One hundred and ninety-five women (44%) were exposed to an estroprogestin drug. The odds ratios (OR) for thromboembolic event were 1.7 [95% confidence interval (CI), 1.2-2.6], 1.8 (CI, 1.2-2.7) and 1.6 (CI, 0.8-3.2) for current hormonal therapy, oral contraceptives and hormonal replacement therapy users respectively. Obesity was associated with an increased risk of thrombosis (OR, 2.1; CI, 1.2-3.6) but smoking was not (OR, 1.2; CI, 0.8-1.9). Second generation contraceptive exposure was associated with a 2.4-fold increased risk of thromboembolic event compared to non-users (CI, 1.4-4.1) whereas third generation products were associated with a 2.0-fold increased risk (CI, 1.0–4.1). Estimated risk for thromboembolic event in current users of second generation oral contraceptives compared with third generation users was 1.2 (CI, 0.5-2.6).

Conclusion: Data obtained from this case-control study show, among women residing in Poitou-Charentes, that combined progestins and estrogens and progestin-only therapy is a significant risk factor of thromboembolic event. Hormonal drug used as contraceptive are associated with a higher risk of event, but hormonal replacement therapy are not. Our data suggest as well that there is no evidence for an increased risk of thromboembolic event with third- compared to second generation combined oral contraceptives.

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Antipsychotic therapy-related drug combinations at risk of QT-interval prolongation or torsades de pointes in the geriatric inpatient population of a teaching psychiatric hospital

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Introduction: Older age belongs to factors that predispose to QT-interval prolongation. In addition, elderly patients frequently have various comorbidities that require multiple drug treatments thereby increasing the probability of potentially deleterious drug interactions. In mental healthcare setting, drug combinations (DCs) at risk of QT prolongation and torsades de pointes may especially occur in case of polypharmacy with QT-prolonging antipsychotics (QT/APs) or concomitant use of QT/APs and bradycardia- or hypokalemia-inducing nonpsychotropic drugs. The aim of the present study was to evaluate the prevalence of these DCs at risk of TdP in the geriatric inpatients (GPs ≥65 year) of a 415-bed teaching psychiatric hospital.

Methods: The study consisted of a 1-day cross-sectional evaluation of all the ongoing drug regimens received by the GPs of the hospital, repeated on two separate days 1 year apart. Drug treatments were retrieved from a computerized physician drug-order entry system and data from both study days were pooled for statistical analysis. The screening/rating tools used by the pharmacy department for reviewing drug regimens with respect to the risk of TdP were the drug product labels and the knowledge base of the French Agency for Medicinal Products. Hazardous or contraindicated DCs (H/C-DCs) or DCs requiring precaution for use were considered.

Results: In total, 152 GPs were included: 102 received antipsychotic therapy of whom 52 were treated with QT/APs (prevalence 51%, 95% CI 41.3-60.7). In this QT/AP-receiving GP group, 36 cases of QT/AP polypharmacy or QT/AP-containing DCs at risk of TdP were recorded, giving a global prevalence rate of 69.2% (95% CI 56.5-81.7).There were eight cases of H/C-DCs due to QT/AP polypharmacy or combinations of QT/APs with amiodarone. QT/AP polypharmacy and combinations of QT/APs with bradycardia- or hypokalemia-inducing nonpsychotropic drugs accounted for, respectively, 22.2% and 77.8% of DCs at risk of TdP. Prevalence of orders for nonpsychotropic drugs potentiating the risk of TdP was similar in the GP group treated with QT/APs and in the GP group receiving other antipsychotics (i.e., nonQT-prolonging antipsychotics), respectively, 53.8% and 38% ($P = 0.22$).

Conclusion: QT/AP prescribing in GPs appeared to be common in our hospital, around 50% of antipsychotic-receiving GPs. Prevalence of antipsychotic therapy-related DCs at risk of TdP was particularly high, nearly 70%, in the QT/AP-receiving GPs, and utilization of nonpsychotropic drugs potentiating the risk of TdP was not minimized in this GP group as compared with the GP group receiving nonQT-prolonging antipsychotic therapy.

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Persistence of statin treatment in real life setting

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Introduction: There is doubt concerning the persistence rate of statin treatment in real life setting. The aim of this study was to assess characteristics and persistence rate of statin treatment.

Methods: We performed a historical cohort study using data from the French social security insurance database (CNAM-TS) of the Aquitaine region between the 1st of March 2004 and the 30th of June 2006. Eligible patients were those registered in the CNAM-TS database from the 1st of January 2004, had submitted for reimbursement a first prescription for a statin between the 1st of September 2004 and the 31st of December 2004 (inclusion period), and did not receive any statin for 6 months before the index date (date of first reimbursement). Available data were patient's demographic characteristics, prescriber type and prescribed drugs submitted for reimbursement. Inside this cohort, four groups were defined as a proxy to define different groups at risk: diabetic patients (DP): submitted reimbursement for antidiabetics concomitantly with a statin; cardiovascular patients (CP): submitted reimbursement for cardiovascular treatments; patients with diabetes and cardiovascular diseases (PDC) and patients without diabetes or cardiovascular diseases. The latter were considered as patients treated for primary prevention.

Persistence rate of statin use was assessed for each group. Discontinuation of the treatment was defined as the absence of any submitted statin reimbursement for 90 days. A switch between statins or a dosage change was not considered to be a treatment discontinuation. Kaplan–Meier analysis was used to estimate persistence rate.

Results: We identified 16, 307 new users of statins (M/F sex ratio: 0.92) representing 13% of the whole population of treated patients in 2004. The median age was 59 years (±13.5) for men and 63 years (±13.8) for women ($P < 10\text{-}4$). Pravastatin was the most prescribed drug and general practitioners the most numerous prescribers (67.1%). CP represented 46% of the study population, 42% were treated for primary prevention, 9% were PDC and 3.6% were DP. The persistence rate at 15 months was 62.3% in primary prevention compared to 78.9% to 83.2% in other groups.

Conclusion: Persistence decreases early in the statin treatment and is higher for patients with few other cardiovascular risk factors.

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Statin utilisation patterns: knowledge of patients and adherence to treatment

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Introduction: Several studies performed on administrative databases have shown bad rates of adherence (persistence) to statin treatment but only a few studies have investigated the reason particularly with regard to the patients who are nevertheless the main actors. The aim of this study was to describe the prescription patterns, use of statins and adherence to treatment regimen, patients' comprehension of the treatment.

Methods: Seventy hospitalized patients treated with statins were interviewed. Several aspects were investigated: demographic and medical characteristics of the patient, treatment patterns, knowledge and perception of the treatment by the patient, the physician/patient relationship and adherence to treatment regimen.
Results: The male/female sex ratio was 0.8 and the median age was 76 years for women and 66 years for men (extremes: 45–93). Prevalence of cardiovascular risk factors was high (systemic hypertension: 73%, diabetes mellitus: 27%, cardiovascular history: 30%, familial cardiovascular history: 57%, BMI > 25: 59%).
Fourteen per cent of the patients had no knowledge of their treatment by statin. Among those having knowledge of their treatment, 20% knew that this treatment was for hypercholesterolemia, or in the prevention of cardiovascular diseases and 70% for treating cholesterol. 21.7% of the patients knew that the objective of monitoring cholesterol levels was to limit the risk of myocardial infarction or stroke and 45.7% answered that the objective was to limit "the narrowing of arteries". Only 48.3% of the patients estimated that they required this treatment, 51.7% thought that it was effective while 73.3% declared that their physician had been right to prescribe them this drug. One third of the patients answered not to have received information regarding their treatment. A diet was followed only in 58.3% of the cases and only 26.7% declared a physical activity. Fifty per cent of the patients considered that their physician had explained to them their pathology and its consequences, and 56.7% estimated to have received from their physician sufficient information concerning their treatment. 13.2% of the patients were found to be non–adherents. The main reasons given were tiredness with regard to daily taking and departure for a holiday.
Conclusion: This study allowed the assessment of adherence by interviewing patients which is rarely performed. The results show the importance of the relation between the physician and the patient, and that of the patient's role in the management of the treatment. Better understanding of these factors is important to be able to include them in decisions regarding medical practices.

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Polymedication in a French community-dwelling elderly population: data from the 3C study

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Introduction: Older persons have an increased number of comorbidities and therefore use a greater number of medications. The aims of this study were to describe the number of medications taken regularly in a community-dwelling elderly population according to socio-demographic characteristics and medical conditions and to identify the factors associated with increasing use of medications.
Methods: The study population sample comprised 9294 subjects aged 65 years or older who were recruited from three French cities (Bordeaux, Dijon, and Montpellier) between 1999 and 2000. Medication use, medical conditions and socio-demographic characteristics of the subjects were obtained at home by face-to-face interviews. Medication use was described in terms of the number of different medications. The level of medication use was categorized as: none, low (1–2), moderate (3–4), high (5–6) and very high (≥7). Univariate and multivariate logistic regression models estimated the association for independent variables in the model with an ordinal polychotomous dependent variable for an increasing number of medications (Odds Ratio and 95% confidence intervals). Backward elimination strategy was used to obtain the optimal set of independent variables.
Results: More than 91% of the sample regularly took at least one medication. The mean number of medications taken was 4.8 (SD: ±2.7). The categories of medications mainly used were cardiovascular drugs (85.4%) and psychotropic drugs (31.1%). Independently of the study centre (Bordeaux, Dijon or Montpellier), increased use of medications was associated with gender (women vs. men, *OR* = 1.8; 95% 1.6–1.9), age (vs. 65–69 years: OR70–74 = 1.1; 1.0–1.2, OR75–79 = 1.3 1.2–1.5 and OR ≥ 80 = 1.2; 1.0–1.4), congestive heart failure (1.9; 1.5–2.3), diabetes (2.4; 2.0–2.8), heart disease (3.0; 2.6–3.5), lung disease (1.5; 1.3–1.7), stroke (1.7; 1.4–2.1) and hypertension (2.0; 1.8–2.2). It was also associated with depressive symptomatology (1.5; 1.3–1.7), disability (vs. grade 0: OR grade1 = 1.4; 1.3–1.5, OR grade2 = 2.1; 1.8–2.3 and OR grade3 = 2.7; 1.6–4.6), self-rated health (vs. high, OR medium = 1.8; 1.7–2.0 and OR poor = 2.6; 2.1–3.3).
Conclusion: Our results suggest that individual characteristics and medical conditions may help identify older persons at high risk for using an increased number of medications.

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Trends in prevalence of potentially inappropriate medication use in older in the East of France: Comparison of two explicit criteria

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Introduction: A potentially inappropriate medication is a medication that is associated with greater risk than benefit and can generally be replaced by a safer alternative. Criteria for potentially inappropriate medications use among older patients have been used in the past decade to identify risk management strategies and preventable medical errors.
The aim of this study was to assess trends in the prevalence of potentially inappropriate medications use in older people from 1995 to 2004 using the 1997 Beers criteria and Lechevallier criteria (a French update of the Beers Criteria).
Methods: We performed a retrospective population-based cross-sectional study using data collected among people aged at least 65 years, examined in three centers for preventive medicine located in the east of France (Vandoeuvre, Verdun and Longwy) from 1995 to 2004. Participants were healthy elderly. Studied variables were sociodemographic characteristics, clinical data and medication use. Data concerning medication use were collected during face-to-face interviews by trained nurses. Each pharmaceutical preparation used during the 2 days before the interview in long run (more than three months) was registered. During the interview, the nurse used the prescription forms and drug package provided by participants to collect data. The main outcome measures were the overall annual prevalence of potentially inappropriate medication with the two explicit criteria: 1997 Beers criteria and Lechevallier critera.
Results: A total of 30 683 examinations were analyzed during the study period. Most examinations were made by females (51.2 %). The mean age was 70.1 (4.3) years. The annual overall prevalence of potentially inappropriate medication use decreased over the time (38.5% in 1995 to 24.0% in 2004; $P < 0.0001$ using Lechevallier criteria, and 17.1% to 11.4%; $P < 0.0001$ for 1997 Beers criteria for the same period. The annual prevalence use of cerebral vasodilators use differed between the two explicit criteria. This prevalence decrease from 25.3% to 13% using Lechevalier list, and from 5 to 2.2% with the Beers list. Potentially inappropriate medication use increased with age [1.06 (1.05–1.07); 95% CI], and more observed in female gender [*OR* = 1.83 (1.74–1.93); 95 % CI] using Lechevallier list.
Conclusion: The prevalence of potentially inappropriate medication observed with Lechevallier criteria was higher. This can be due to the fact that the list of medications considered by this author was more extensive than the Beers' list. The decrease of the prevalence use of cerebral vasodilators observed in this study was consistent with furthers studies in France. This study suggested that potentially inappropriate medication was strongly dependent on the list used and decreased over time.

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First results of the OSIAP Europe Project: pharmacoepidemiological survey of misuse or abuse of marketed drugs

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Introduction: The French network of Centres d'Evaluation et d'Information sur la Pharmacodependance (CEIP) performs since 2001 a prescription forgeries survey with community pharmacies networks. The system called OSIAP (Ordonnances Suspectes Indicateur d'Abus Possible) provides information about potential abuse liability of marketed drugs in France. The collection data is based on voluntary pharmacies participation. We began in January 2005 a European collaborative project funded by the 2003–2008 Public Health Program of the European Commission, to extend the OSIAP system in five European countries. The aim of this project is to compare spatial and temporal trends of marketed drug diversion and to assess the impact of regulatory measures. In parallel, we index the drugs for which the potential of abuse is known or suspected, to compare, according to the countries, the specialities and dosages available, as well as the scheduling and rules of prescription in each country.
Methods: The participants developed common methodological tools (for data collection and criteria for identification of suspect prescriptions) and performed a voluntary pharmacies network in one (at least) area of their country, in order to collect suspect prescription forms during 2 months/year.
Results: The first collection data was done in May 2006. Around thirty pharmacies participated in Belgium (covering 150 000 inhabitants), Italy (100 000), the Netherlands (300 000), Spain (150 000) and around 900 in France (2.4 millions) and Sweden (14 millions inhabitants). Pharmacists identified 169 suspected prescription forms for France, 13 for Belgium, 33 for Spain, one for Italy. The suspicion criteria most reported by Belgian and Spanish pharmacists was the non-respect of prescription rules. The medicines the most often reported were flunitrazepam (Belgium), alprazolam, methylphenidate and clorazepate, (Spain) bromazepam, zolpidem and buprenorphine (France). Most of reports concerned narcotics (or drugs with similar scheduling) in the Belgium collection, three of these drugs being not marketed in France.
Conclusion: Three periods of suspect prescriptions collection are expected before end of 2007, allowing a European comparison of marketed drug diversion. Because drug utilization patterns may be different between all countries (Belgium is for example one of the European countries with the highest level of narcotic use) it will be interesting to compare results of prescription forgeries survey and level of use.

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Characteristics of an uncontrolled hypertensive patients population: a cross sectional study

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Introduction: Cardiovascular prevention is now based upon the global cardiovascular risk assessment and management of the population. In a French cohort of hypertensive patients (Centre d'Investigations Préventives et Clinique d'Ile-de-France), 87% of men and 80% of women presented at least another associated risk factor (in normotensive controlled patients, these proportions were respectively 64% and 54%). The associated major risk factors were, by order of decreasing frequency: smoking, 33.4%, hyperlipidemia, 29.6%, tachycardia, 24.2%, obesity, 14.4% and diabetes, 8.5%.
Objective: To describe the characteristics of uncontrolled hypertensive patients, in particular the association with other cardiovascular risk factors (CVRF).
Methods: Cross-sectional observational study carried out according to the methodology ORP® (MediSCAN): 1053 general practitioners randomly selected collected, among 6688 all coming hypertensive patients, the characteristics of 1736 uncontrolled hypertensive patients. During the collection of the data, missing or incoherent informations were the subject of requests. Totally 5.4% of the patients recently diagnosed were excluded from the analysis.
Results: The patients with uncontrolled blood pressure level were in majority males (56%) aged 61 years on average and have been followed for more than 5 years. Totally 17.2% did not receive antihypertensive drugs; 2/3 were in overweight or obese (BMI ≥ 26); 44% had only one another CVRF; 56% had two or

more. The CVRF were hyperlipidemia (51%) (86 of which were under treatment), sedentariness (43%), obesity (33%) and current or past smoking (28%). Despite the frequent association of CVRF in the studied population, 81% of the patients had been treated only for one CVRF. The quality of control of the blood pressure levels were related to the number of the CVRF. Antihypertensive drugs were used in monotherapy by 25.6%, bitherapy by 58% and tritherapy by 16%. The drugs used were inhibitors of the renin-angiotensin system (66%), calcium antagonists (10%), beta blockers (10%), diuretics (17%), others (6%).
Conclusion: This study highlights the importance of the insufficient control of the blood pressure and the undermanagement of associated CVRF in hypertensive treated patients.

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Drugs in general practice: patients' expectations, doctors' perceptions and related-behaviour – a questionnaire survey
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Introduction: In France, a large majority of medical consultations ends with a prescription of medication. Previous studies have revealed that these prescriptions are not always strictly related to pure medical grounds. The aim of this work was to assess patients' expectations from consultations and their perceptions by general practitioners, in order to understand some factors explaining over-prescribing.
Methods: The questionnaire survey was carried out in the south west of France (Midi-Pyrenees) between January and March 2006. The sample consisted of 27 randomly selected general practitioners and 1862 patients. During an interview, doctors answered several questions about their orientation towards drugs. Self-administered questionnaires were also completed by consenting adult patients waiting for a consultation with their general practitioners.
Results: Diagnosis appeared to be the major expectation for 56% (1023/1824) of participating patients, treatment for 32% and medication for 5%. Eighty eight per cent (1599/1827) of those patients reported that they did not expect a prescription for each consultation. When doctors decided not to prescribe any drug, 83% (1532/1836) of patients agreed with this decision. If none drug was prescribed, several proposals were made to patients: further investigations (47%), waiting a few days and coming back if necessary (43%), referral to a specialist (28%). Analysis of influence of socio-demographic characteristics showed that a greater proportion of people in older age groups expected a medication. No statistically significant differences were found between sex groups.
Doctors putted the focus on listening to the patient (15/27) but 41% (11/27) of them tended to say that patients expect a drug at the end of each consultation. When doctors considered that no drug was required, whereas patients expected one, 41% (11/27) of doctors suggested their patients using complementary therapy, 4% (1/27) prescribed the excepted drug and 26% (7/27) prescribed it if patients insisted.
Conclusion: We showed that there was a difference between patients' expectations and physicians' perceptions of these expectations. This misunderstanding could be partly responsible for inappropriate drug prescribing. However, patients still trust their doctor and listening might be the first step to a useful common dialogue. General practitioners could use this new relationship to improve health care and give other answers that the pharmaceutical one to multiple patients' expectations.

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Analysis of pharmaceutical dispensation in patients chronically treated with drugs registered in the generic drug list
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Introduction: Since 1996, the generic market has increased. At the present time, in the French Department 'Maine et Loire', the rate of generic dispensation is around 70% for drugs of generic list. Because of generic substitution laws and measures aiming to increase generic competition, fear is high to observe drug misuses because of multiple switches to different drug presentations of a given active principle (PA) in long-term treated patients. The objective was to measure the numbers of different dispensed drug presentations (DDP), and of switches observed in outpatients during a 1-year treatment with drugs of generic list.
Methods: The study was observational and used computerized records of the general health insurance system (CPAM) of Angers. Four AP were selected for their long-term use and/or their narrow therapeutic index: carbamazepine, buflomedil, metformine and paroxetine. For each AP, requests on data base between 2004/06/01 and 2005/05/31, provided the following data: number of treated patients and for each of them: age class, number and date of dispensing acts, number of prescribers, number of dispensing pharmacies, number of DDP (including brand name and generic drugs) and number of switches. Patients with <11 and more than 14 dispensing acts during the study period were excluded.
Results: Carbamazepine was used in 389 patients (median class age: 50–54) as generic drugs in 24% of all dispensing acts. The mean number of DDP was 1.4 (1–3) with a median at 1. The mean number of switches was 0.84 (0–10) with a median at 0. Buflomedil was used in 503 patients (median class age: 70–74) as generic drugs in 81% of all dispensing acts. The mean number of DDP was 1.6 (1–6) with a median at 1. The mean number of switches was 1.1 (0–11) with a median at 0. Metformine was used in 2741 patients (median class age: 65–69) as generic drugs in 69% of all dispensing acts. The mean number of DDP was 1.8 (1–6) with a median at 1. The mean number of switches was 1.3 (0–11) with a median at 0. Paroxetine was used in 1189 patients (median class age: 55–59) as generic drugs in 60% of all dispensing acts. The mean number of DDP was 2 (1–6) with a median at 2. The mean number of switches was 1.8 (0–12) with a median at 0. The risk factors to observe 2 or more switches in the course of the treatment were a number of 2 or more dispensing pharmacies, and the fact to be given at least one time the brand name drug. The number of prescribers did not impact the number of switches. Age ≥ 65 years did not protect the patients from multiple switches.
Conclusion: In the great majority of patients, who used a single pharmacy, the numbers of DDP and of switches are less than two and finally the risk of misuses is probably less than was feared.

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Switching from traditional non-steroidal anti-inflammatory drugs to Coxibs in the CADEUS study
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Introduction: Using data from the CADEUS study, we studied the frequency of switches from traditional non-steroidal anti-inflammatory drugs (tNSAIDs) to Cox-2 inhibitors and identified the characteristics most likely to be associated with switching.
Methods: Between July 2003 and August 2004, 13 553 subjects affiliated to the French National Health Insurance System for salaried persons (CNAM-TS) who had a claim for tNSAIDs (date of claim = index date) were included in CADEUS. This study was performed on a sub-population of the cohort, composed of subjects without any Cox-2 inhibitor claim during the 6 months preceding the index date and suffering from rheumatismal pathology. A switch was defined as at least one Cox-2 inhibitor claim during the 6 months following the index date. For each included subject, socio-demographic, medical and drug delivery data were considered. Characterization of switchers was performed using logistic regression models.
Results: Of 13 553 tNSAIDs users in the CADEUS study, 5230 were included had no Cox-2 inhibitor claim over the 6 months preceding index date. During the 6 months following the index date, 433 (8.2%) had at least one Cox-2 inhibitor claim. Compared with non-switchers, switchers were older (OR 10 yrs = 1.1; 95% CI: 1.01–1.2), less likely present with inflammatory rheumatism (OR = 0.4; 95% CI: 0.2–0.7) and more likely to be former users of gastroprotective agents (OR = 1.7; 95% CI: 1.3–2.3).
Conclusion: Switch from tNSAID to Cox-2 inhibitor concerned <10% of subjects. Switching is most important in subjects known to be at risk of gastrointestinal adverse events.

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Misuse, abuse and dependence of psychotropic drug in France – From OPPIDUM program of CEIP Network
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Introduction: Psychotropic drugs consumption in France is the most important of Europe. Among different psychotropic drugs, some of them can be abused. Different information concerning consumption of psychotropic drug has been studied in OPPIDUM Program to 2003 until 2005.
Methods: The Oppidum Program (Observation of illegal drugs and misuse of psychotropic medications), a multicentric survey, annually surveys drug dependent subjects attending specialized care centres throughout France. Data were collected by questionnaire on socio-demographic variables and drug use during the preceding week. Different classes of psychotropic drug have been studied: benzodiazepines (BZD), other anxiolytics or hypnotics, antidepressants and neuroleptics.
Results: The abuse potential is highest with the classes of BZD. Among them, flunitrazepam still the most abuse: 83% of daily dose was superior to the recommended daily dose, 85% of description of abuse or dependence and 57% of flunitrazepam was obtained illegally. Clonazepam is also abused: 31% of clonazepam was obtained illegally, 68% of description of abuse or dependence. Some data suggest an abuse of benzodiazepine-like drugs (zopiclone and zolpidem) (24–30% of description of abuse or dependence, 27% of daily dose was superior to the recommended daily dose) and with the other anxiolytics like meprobamate (39% of description of abuse or dependence). Few data suggest an abuse of antidepressants and neuroleptics. Among them, cyamemazine (4% obtained illegally and 21% description of abuse or dependence), venlafaxine (18% description of abuse or dependence, 4% obtained illegally), fluoxetine (14% description of abuse or dependence) and tianeptine (44% daily dose was superior to the recommended daily dose, 15% description of abuse or dependence).
Conclusion: The analysis of this study needs to take into consideration the specific population and can not be generalized to the whole population. Nevertheless these data are precisely interesting due to the characteristics of this population with history of abuse.

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Patients at risk of post-operating nausea and vomiting (PONV): evaluation of a predictive score
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Introduction: Post-operating nausea and vomiting (PONV) are frequent (20–60%) and constitute with pain, the most common cause of failure and patient dissatisfaction in ambulatory surgery. Apfel Score (AS) is currently used to identify patients at risk.[1] A score superior of 1, supposes a high-risk of PONV (40%) and thus a prophylaxis. The objectives of our study were to estimate the incidence of PONV in our university hospital, and to compare the AS to another local decision algorithm.
Methods: An observational one day transversal study has been done at 24-h post-operating. Data were collected from the patient files and by interviews. Data concerned AS, surgical and anaesthetic risk factors and PONV morbidity. All surgical wards were concerned unless paediatrics, obstetrical and ambulatory surgery. We excluded patients unable to answer.
Results: Among patients included, 10% were excluded leaving 133 questionnaires to analyse. Incidence of PONV was 37% (47/133). The number of patients who benefited of a prophylaxis anti-emetic treatment was equal to 11 (8.27%). The treatment failed for 27%, (3/11). Of them, 64% (7/11) had an AS superior or equal to 2. Applied to this population, the AS would have allowed the prophylaxis of 88 patients (66.2%). Predictive positive value (PPV) of the AS was 46% and negative predictive value (NPV) was 80%, whereas with our local algorithm the NPV was equal to 90% and the PPV would have been constant (42%). With this algorithm 111 patients would have been treated (83%) instead of 88.

Conclusion: In our university hospital, the prophylaxis of PONV is ineffective because insufficiently applied. The use of the AS could improve the management of PONV but seems to be insufficiently sensitive. We have initialized a work on the risk factors that are integrated within our local algorithm to optimize its specificity.

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Clinical description and therapeutic management of tuberculosis in patients co-infected with the human immunodeficiency virus (HIV)

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Introduction: Therapeutic management of tuberculosis, the most frequent opportunistic infection during HIV disease, is challenged by drug toxicity, drug–drug interactions with HAART, bacterial resistance and treatment adherence.

Methods: We retrospectively described between 1996 and 2004, the clinical and management characteristics in a population of 111 HIV-infected patients at various stages of the disease with concomitant tuberculosis with bacterial and/or histological confirmation. Besides demographic characteristics, we particularly focused on the time to HAART introduction, type of HAART, drug toxicity reported and the incidence of resistant bacterial strains.

Results: Among the 111 patients retrospectively assessed (72 men and 39 women, mean age of 38 years, mean CD4 count of 191/mm^3, mean plasma viral load of 6.2 $\log_{10}$ copies/ML), 77 of them (69%) were from Sub-Saharian Africa. In 48% of cases (n = 53), tuberculosis revealed the HIV infection. Among the 74 patients with bacterial documentation, 10 had resistant strains (14%), mainly to isoniazide (n = 8), to ethambutol (n = 1) and to isoniazide and rifampicine (n = 1). Among the 53 patients in whom tuberculosis revealed HIV infection, HAART was introduced in 44 cases 2.8 months upon tuberculosis diagnosis and mainly included two nucleoside analogues (NA) and either nevirapine in 20 patients (37% of cases) or a ritonavir-boosted protease inhibitor (BPI) in seven cases (13% of cases). In nine patients, HAART was not started due to prolonged anti-tuberculosis agents-related toxicity. In the remaining 58 patients with known HIV infection prior to tuberculosis, 33 (57%) already received HAART, that was maintained in 18 of them (54%) and included two NA (n = 6), two NA with nevirapine (n = 6) and two NA with a BPI (n = 4). In the remaining 15 patients, HAART was resumed 2.6 months upon antibiotic treatment start. Drug-related adverse events occurred in 41 patients (37% of the total population) and included cytopenia (n = 9), hepatitis (n = 16, i.e. 14% of the whole population), nausea and diarrhoea (n = 4), skin rash (n = 4), kidney failure with hypokalemia (n = 4) and peripheral neuropathy (n = 4). Among the 16 patients with liver toxicity, only 7 (i.e. 6% of the total population), were attributable to anti-tuberculosis agents while no liver toxicity was observed in patients receiving nevirapine.

Conclusion: Management of tuberculosis in HIV-positive patients did not differ from that observed in the HIV-negative population. Although the therapeutic combination with HAART was challenged in more than 20% of patients in the current cohort, anti-tuberculosis agents-related liver toxicity was similar to that observed in a recent cohort of 715 HIV-negative patients with tuberculosis (1).

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Initial treatment effectiveness of acute sinusitis in representative national cohort

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Introduction: A pharmacoepidemiological study was performed to assess the effectiveness of the initial treatment for acute sinusitis in current medical practice in France.

Methods: A prospective observational cohort study of patients with acute sinusitis was carried with a representative sample of general practitioners (GPs) and ear–nose–throat specialists (ENTs). Patients diagnosed with acute sinusitis were included in two 4-week periods (March/April and September/October 2005). Effectiveness of the initial treatment was evaluated by the absence of failure (sinus drainage or new antibiotherapy or stop of treatment for side-effect) within the 10 days following inclusion, and the lack of a relapse/recurrence (sinus drainage or initiation of new antibiotic) between the 11th and 60th day. Cox proportional hazard model was used to identify prognostic factors of failure.

Results: One thousand, one hundred and seventy-four GPs and 120 ENTs included a total of 5693 patients. The mean age of the patients was 42.5 years, 60.2% were women and 32.3% had a history of sinusitis in the previous year. Localizations were maxillary (47.7%), ethmoido-frontal (28.5%), pansinusitis (22.8%) and sphenoid (0.7%). Antibiotics were prescribed to 92.4% of the patients. Analgesics, corticosteroids, NSAIDs and others symptomatic medicines were prescribed to 50.1%, 48.8%, 18.0% and 44.7% of the patients respectively. Absence of treatment failure at 10 days varied from 91.8% to 95.8% for the various antibiotics and was 86.4% in patients not prescribed antibiotics. Lack of relapse/recurrence at 2 months varied from 85.6% to 90.4% for the various antibiotics and was 80.8% in patients not prescribed antibiotics. Compared with patients not prescribed antibiotics, the relative risk of failure varied from 0.28 to 0.58 for the various antibiotics, after adjustment for patient characteristics and associated treatments. For patients without treatment failure within the 10 first days, the risk of relapse/recurrence at 2 months did not differ between patients prescribed antibiotics or not.

Conclusion: Initial antibiotic prescription for the treatment of acute sinusitis reduced the risk of failure at 10 days 1.7 to 3.6-fold, but had no effect on the risk of relapse/recurrence at 2 months.

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Management of acute sinusitis: result of a French cohort study

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Introduction: A pharmacoepidemiological study was performed to describe the management of acute sinusitis by general practitioners (GPs) and ear–nose–throat specialists (ENTs) in France.

Methods: A prospective observational cohort study of patients with acute sinusitis was carried with a representative sample of general practitioners (GPs) and ear–nose–throat specialists (ENTs). Patients diagnosed with acute sinusitis were included in two 4-week periods (March/April and September/October 2005). Statistical descriptive analysis was weighted in order to take into account acute sinusitis activity of practitioners and the national distribution of GPs and ENTs.

Results: One thousand, one hundred and seventy-four GPs and 120 ENT included 5238 and 455 patients respectively. Compared with national statistics, the sample of GPs and ENTs was representative with respect to geographic distribution and age, although women were under-represented (21.3% vs. 27.7% nationally). The mean acute sinusitis case load of GPs and ENTs was respectively 10.8 and 10.4 cases/month (median 8.4 and 6.7). Extrapolation to 57 988 GPs and 2271 ENTs in France allows to estimate that about 97% of patients with acute sinusitis are seen by GPs vs. 3% by ENTs. The mean delay between the onset of symptoms and inclusion was 4.7 days (4.5 for GPs and 8.6 for ENTs). Localizations were maxillary (47.7% for all patients, 47.2% for GPs and 60.6% for ENTs), ethmoido-frontal (28.5%, 29.1% and 12.0% respectively), pansinusitis (22.8%, 22.7% and 24.4% respectively) and sphenoid (0.7%, 0.7% and 1.6% respectively). Antibiotics were prescribed to 92.4% of the whole study population (GPs 92.4%, ENTs 92.7%). Antibiotics prescribed most frequently were cefpodoxime 18.3%, co-amoxiclav 17.7%, telithromycin 11.9%, cefuroxime 10.4%, pristinamycin 9.1%, moxifloxacin 8.0% and levofloxacin 3.3%. The most frequently co-prescribed drugs were analgesics (50.1% for all patients, 51.4% for GPs and 14.5% for ENTs), corticosteroids (48.8%, 48.4% and 61.9% respectively), symptomatic medicines (44.7%, 45.5% and 19.8% respectively) and NSAIDs (18.0%, 18.4% and 7.5% respectively). A sinus X-ray was prescribed to 9.7% of patients (9.5% for GPs and 14.0% for ENTs).

Conclusion: Most of patients with acute sinusitis (97%) were treated by GPs. There were minor differences in patient characteristics and management between GPs and ENTs.

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A comparative study about factors associated with QTc prolongation according to methadone exposure: role of different drugs

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Introduction: Recent case series suggest the role of synthetic opioïd methadone in QT prolongation and Torsades de Pointes (TdP) ventricular arrhythmia. We previously presented the preliminary results of a study investigating the frequency of prolongation of QT interval in patients treated by methadone for opiate maintenance, in which a comparison with controlled patients not exposed to methadone was planed, but not done. We found a significant relationship between prolongation and methadone, but most of patients were also exposed to several drugs known to induce QT prolongation, for example neuroleptic drugs. Thus, the aim of this presentation is to complete these data by comparing results obtained in controlled patients.

Methods: This is a controlled cross sectional study in which patients exposed to methadone recruited from addiction centres and from hospital units were compared to controls not exposed, selected from hospital psychiatric unit and matched on co-prescribed drugs. A standard 12-lead electrocardiogram was performed for all patients in the same condition. QT interval and QT dispersion were measured and corrected from heart rate using Bazett's method. Data concerning drug exposure and other clinical data were collected from patient's interview and from medical files.

Results: Between December 2004 and December 2006 we selected 42 patients on methadone treatment and 42 controls. Among cases, all patients were treaded by methadone and 18 (43%) were exposed to psychoactive drugs known as prolonging the QT interval. The value of QTc interval was 412 ± 32 ms (range 320–485) and QTc dispersion was 54 ± 33 ms (range 15–145). A prolonged QTc and QTc dispersion were observed in 7% and 12% of the patients. Thirty-nine controls (93%) were exposed to psychoactive drugs known as prolonging the QT interval. One patient was treated by buprenorphine. We will investigate if QTc and QTc dispersion differs in patients according to exposure to methadone or not by a model of multiple linear regression.

Conclusion: Our previous study suggested than methadone could prolong the QTc interval, and several factors were suspected: methadone dosage, co-prescribed drugs, especially CYP 450 inhibitors and some psychoactive drugs, cocaine use and history of heart disease. This comparative study with patients treated by buprenorphine or without opiate maintenance treatment could confirm our results and conclude to a causative relationship between methadone and QT.

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Taking into account the interactions with hormonal contraception: an essential point to avoid unwanted pregnancy

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Introduction: In 2000, 73.6% of the women from 18 to 44 years state to use a contraceptive method. The pill was the most largely used (45%). Hormonal contraception has different ways of administration (pill, intra-uterine device, ring,

and patch). Many drugs interact with the hormonal contraceptives. Some of the interactions are well known, others less, but all can lead to a contraception failure and thus to an unwanted pregnancy.
Methods: A review of the literature is set out from three cases of unwanted pregnancy inspite of hormonal contraception, reported to the Regional Pharmacovigilance Center (CRPV) of Amiens.
Different drug interactions, proved or potential, being the cause of a lack of efficiency, are listed.
Results: First case: interaction between ethyniloestradiol/levonorgestrel and carbamazepin, pregnancy discovered weeks of amenorrhea.
Second case: interaction between etonogestrel (implant) and rifampicin, pregnancy discovered at 25 weeks of amenorrhea.
Third case: interaction between levonorgestrel and bosentan, pregnancy discovered at 15 weeks of amenorrhea.
Review of literature: The mean listed interactions are linked to enzymatic induction but other mechanisms can be involved.
The cases reported to the CRPV were unwanted high-risk pregnancies. In these cases, all the associated drugs were well-known to interact with the contraceptive (mention in the product characteristic summary). They linked to the prescription, during pregnancy of drug known as teratogenic or without guaranty of the absence of teratogenic effects.
Conclusion: It is necessary to be insistent with the physicians on the systematic research of the possible interactions between prescribed drugs and hormonal contraceptive in every patient of childbearing age.
In the case of prescription of potentially teratogenic drug able to interact with hormonal contraceptive patients of childbearing age should be systematically warned against this interaction.

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The drug compliance – a 'random' variable whose taking into account is essential: example of the oral anticoagulants treatment (OAT)
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Introduction: The compliance may be defined as the extent to which the patient follows a treatment prescribed by a physician.
For OAT, the compliance should be 'theoretically' better than with other drugs owning to the particular recommendations connected with the prescription (monitoring of the International Normalized Ratio [INR], previous training of the patient).
Methods: The clinical cases were extracted from a prospective cohort study driven by the Pharmacovigilance Center of Amiens. In this study, hospitalized patient with high INR (≥5) are analysed. The roles of patients and of medical staff as causes of excessive anticoagulation are accessed.
Results: With these clinical cases, was illustrating the fact that the 'actual' compliance is a problem certainly more complicated and more frequent than expected. The compliance or even the low compliance can be a cause of severe adverse drug reaction.
Conclusion: One of the solutions seems to be a repeated training and a consistent assessment of knowledge. The refusal or the non- or the misunderstanding of instructions (planned non-compliance) must lead to the withdrawal of the treatment.

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Allopurinol-induced severe systemic hypersensitivity reaction (DRESS syndrome)
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Introduction: The drug hypersensitivity syndrome, or DRESS syndrome (drug rash with eosinophilia and systemic symptoms), is an uncommon but potentially life-threatening idiosyncratic drug reaction. DRESS is a severe toxiderma accompanied by lethal visceral involvement in 6 to 10% cases. We report herein an observation of a severe DRESS syndrome induced by allopurinol in order to discuss the underlying mechanisms.
Methods: A 74-year-old man exhibiting fever, malaise, pruritic rash skin, as well as continuous coughing with dyspnea, acute myalgia with rhabdomyolisis and abdominal aches, was admitted to the hospital. Two weeks before, allopurinol had been prescribed by the general practitioner because of asymptomatic hyperuricaemia. His health state deteriorated, evidencing abnormal liver enzymes (ASAT 212 U/L, ALAT 280 U/L) and haematological parameters (prothrombin time: 34%). Hepatic cytolysis associated with vesicular lithiasis was diagnosed. Although allopurinol was suspected to cause severe side-effects, it was not halted and the patient was released. One week later, another general practitioner noted the strongly deteriorated state, including fever, icterus and adenopathy. Blood samples showed abnormal liver enzymes (ALAT 474 IU/L, ASAT 350 IU/L), high serum creatinine (171 μmol/L) and leucocytosis (18.1×10^9/L). Allopurinol treatment was immediately stopped and the patient admitted in Cardiology Unit. Hemocults and serological tests (HBV, HCV, HIV, CMV, Epstein-Barr virus, syphilis, Toxoplasma) were negative.
Results: Our observations lead us to a diagnosis of hypersensitivity syndrome considering the characteristics of the patient's condition: (i) clear exposure to allopurinol; (ii) time frame (cutaneous rash 14 days after medication start); (iii) clinical signs, including altered general state with fever, acute hepatocellular injury, worsening renal function, adenopathy, rash and leucocytosis; (iv) lack of exposure to another drug which may have caused a similar clinical picture, a picture that fits the criteria for DRESS syndrome diagnosis. Febrile hepatic failure (cholestatic jaundice, cytolysis, coagulation abnormalities) without aetiology needed research of drug consumption in case of DRESS syndrome. A favourable clinical evolution was observed following allopurinol medication withdrawal. This case points out the importance of an early diagnosis and quick withdrawal of the drug in order to prevent severe complications.
Conclusion: The risk of immediate or delayed serious complications associated with allopurinol use prompts to carefully consider the benefits of its prescription. Totally 52–75% of allopurinol-induced DRESS syndromes were reported in patients exhibiting an asymptomatic hyperuricemia. It is important to recognize this entity recently named DRESS syndrome as it is potentially serious and a fast withdrawal of the drug responsible is thus imperative.

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Adverse drug reactions of methylphenidate in French Pharmacovigilance database
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Introduction: Methylphenidate is a piperidine derivative structurally related to amphetamine, with similar pharmacological properties. Methylphenidate is commonly used to treat attention deficit hyperactivity disorder (ADHD) for children over six. This trouble is the most common psychiatric disorder of childhood and adolescence. The disorder affects 8–12% of children worldwide. Diagnosis and treatment of ADHD are controversial. Neurologists, psychiatrists and paediatrician, only, are allowed to prescribe methylphenidate which is covered by 'narcotics' schedule.
Methods: The study used data from French Pharmacovigilance Database of adverse drug reactions spontaneously reported by health professionals from 1985 until 2006.
Results: Fifty-seven adverse events were reported with methylphenidate of which nine in adults. The most common reported side-effects were neurological and psychiatric (43.8%) followed by skin reactions (7.5%). 48 children are mainly concerned (10 ± 3 year-old), 36 boys and 12 girls (one serious case in a boy of 4 years). Convulsions are the most predominantly reported neurological adverse events ($n = 6$). Five cases of 'unpleasant hallucinations' are reported. Two growth suppressions are observed. Only one alopecia and one thrombocytopenia are notified. In adult, addiction is reported in three cases, off label use in eight cases.
Conclusion: The profile of undesirable effects is similar for the different forms of methylphenidate. The limitation of spontaneous reporting is essential in the assessment of psychiatric adverse events due to the high degree of co morbidity (up to 50%) between ADHD and other psychiatric disorders. It is difficult to draw definitive evidence of a causal link between psychiatric manifestations and methylphenidate administration. The limitations of the data for long term treatment are mentioned in the 'warnings and precautions for use' section of the Summary of Products Characteristics. Long-term studies, over 3 years, are needed.

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A case of serotonin syndrome caused by venlafaxine and lithium
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Introduction: The Serotonin syndrome is a toxic hyperserotoninergic state, resulting from the hyperstimulation of the brainstem and spinal cord 5-HT1A-receptors, which is caused by the interaction between a serotoninergic agent and a serotonin-enhancing drug. This syndrome is rare but may be highly serious and compromise the vital prognosis. We report herein an original observation of a patient who suffered from a serotonin syndrome secondary to a co-medication with lithium and venlafaxine in order to discuss the underlying mechanisms.
Methods: A 65-year-old man that had been treated for 24 months by lithium therapy for a bipolar disorder concomitantly received venlafaxine for a major depressive episode. Immediately after a venlafaxine posology increase, he developed several symptoms: dysautonomic and neuromuscular disorders, consciousness and behavioural problems.
Having excluded all other likely causes for the clinical picture observed, in particular a lithium over dosage, a serotonin syndrome was postulated. As to the responsibility of venlafaxine, we note that prior to treatment instauration, no symptoms were observed. The syndrome was resolved completely after the interruption of venlafaxine administration. Lithium therapy was carried on without noticeable problem. An objective causality assessment revealed that adding venlafaxine to the lithium treatment was the most likely cause of the adverse reaction observed.
Results: Serotonin syndrome is a serious adverse reaction usually due to interactions with serotonergic drugs. This syndrome is most commonly seen in patients for whom two combined agents increased serotonin availability by two separate ways. In our clinical case, we have two drugs increasing the serotoninergic activity in the central nervous system: an agonist of serotonin and dopamine receptors (lithium) and an inhibitor of serotonin reuptake (venlafaxine). Various mechanisms may raise the serotonin (5-hydroxytryptamine [5-HT]) concentration: increase of 5-HT release, 5-HT reuptake inhibition, 5-HT metabolism inhibition or post-synaptic receptors stimulation. The commonly held physiopathologic hypothesis is an excessive stimulation of the post-synaptic 5-HT1A-receptors, associated with a central hyper-serotoninergic state.
Conclusion: Because venlafaxine is a potent serotonin agonist, caution must be exercised to prevent the hazard of serotonin syndrome when used with other serotonin agonists. The serotonin syndrome is a potentially severe state that may degrade very fast. It is thus very important to possess a good knowledge of serotonin reuptake inhibitors, both selective (SSRI) and non-selective, to monitor medically a SSRI and lithium association and to quickly stop the treatments if serotonin syndrome symptoms appear.

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Loss of sight: a side-effect of bortezomib?
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Introduction: The proteasome pathway plays an essential role in the degradation of intracellular proteins. Bortezomib is a novel cytotoxic agent that potently and specifically inhibits the proteasome function and it is now becoming commonly used in haematological malignancies.
Methods: Case report.
Results: We report a case of a 60-year-old man diagnosed with follicular lymphoma in 2004. He relapsed two times and received various chemotherapy regimens that were all combined with rituximab. On January 2006, the patient received for his third relapse of B-cell lymphoma, a single antineoplastic agent, bortezomib. The chemotherapy regimen was the following: intravenous bortezomib at 1.5 mg/m^2 at days 1-, 4-, 8-, 11-, on a 21-day cycle basis. At the beginning of

March 2006, because of a severe thrombocytopenia, it was decided to stop definitively bortezomib and the patient was switched to rituximab monotherapy. At the end of March, the patient developed a peripheral polyneuropathy together with a sudden central scotoma of the left eye. During the following week, he developed a sudden loss of sight (amaurosis) of the left eye without diplopia and vertigo. The loss of sight worsened and extended over the right eye. The cerebral magnetic resonance imaging revealed no particular abnormalities and the lumbar punction excluded lymphomatous meningitis. The ophtalmologic examination showed a total blindness (0/10) of the left eye and partial blindness of the right eye (0.8/10) caused by a macular oedema. The patient's past medical history included a surgery of a traumatic extradural haematoma 31 years before. As no concurrent disease could explain the amaurosis, a drug-related toxicity was suspected. As bortezomib was strongly suspected, it was decided to maintain rituximab therapy. Patient recovered partially and spontaneously from his loss of sight. On November 2006, it was stated that the patient had recovered with sequelae as his retina was definitively damaged.
Conclusion: Here, we report a case of a loss of sight that occurred in a patient who received two antineoplastic agents: rituximab and bortezomib. Rituximab could be reasonably excluded as the patient recovered under rituximab therapy and moreover never experienced such side-effect during his previous rituximab treatments. Furthermore, rituximab is a potential treatment for patients suffering from optic neuropathy. Regarding bortezomib, patient developed concomitantly with amaurosis, a peripheral neuropathy. The latter is a well documented side-effect of the drug. This observation strongly suggests that bortezomib could be involved in the genesis of amaurosis, the mechanisms of which remain to be determined.

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Tumour necrosis factor-alpha antagonist, etanercept and demyelinating disease: analyse of French database and review of the literature
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Introduction: The tumour necrosis factor-α (TNF-α) inhibitors are approved for the treatment of moderate to severe chronic psoriasis, rheumatoid arthritis, juvenile rheumatoid arthritis, psoriasis arthritis and ankylosing spondylitis. An increased risk of demyelinating disease is suspected with etanercept, several publications have described ten cases all around the world. We studied the data collected by French pharmacovigilance concerning demyelinating disease and etanercept. The aim of this study is to describe these effects and to explore the relation of etanercept to demyelanating disease.
Methods: Reports were selected in the French Pharmacovigilance database according to the presence of specific Adverse Drug Reaction Terminology (ADR term) with relationship to central and peripheral nervous system. Causal relationship between etanercept and adverse drug effect (ADE) is assessed using the French Pharmacovigilance method of drugs imputability. We investigated indication, patient's demographic data and the prescription (dosage, duration of treatment).
Results: We collected in the French pharmacovigilance database 43 notifications. After analysis, we kept 21 notifications where etanercept is considered 'suspect' according to the OMS score. These notifications concerned 16 women and five men; the middle-age is 51.7 years (13 – 73). Sixteen of the 21 patients were treated for rheumatoid arthritis, two for ankylosing spondylitis one for psoriasis, one for juvenile rheumatoid arthritis and one for pyoderma gangrenosum. The treatment duration was known for 18 patients and the middle was 8 months. In most of these cases, etanercept was administered subcutaneous 25 mg twice a week. The middle duration between the beginning of the treatment and demyelinating disease occurrence was also 8 months. For 10 patients, evolution was known after dechallenge: fast improvement was observed in seven patients.
Conclusion: Physicians must screen candidates for TNF-α antagonist therapy carefully to exclude those with symptoms, signs or with family history of demyelinating diseases. Anyway, prescribers must watch over their patients and stop the treatment in the case of a demyelinating occurrence. Indeed, after discontinuation the drug patients had complete or only partial resolutions of symptoms. Prescribers have a keen awareness of this possible adverse effect given the increased use of this class of drugs.

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Hypersensitivity to bortezomib: a case report
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Introduction: The proteasome pathway plays a significant role in neoplastic growth and metastasis. Bortezomib is a first-in-class reversible inhibitor of the 26S proteasome, which degrades proteins involved in cell cycle regulation and cell signalling.
Methods: Case report.
Results: We report a case of a 64-year-old man diagnosed with follicular lymphoma in 1997, with relapses on 2001 and on 2005. For this later relapse, the patient was treated with a single antineoplastic cytotoxic agent; bortezomib. The study medication was as follows: a 21-day cycle of 1.5 mg/m^2 bortezomib twice weekly for 2 weeks. Three days after the third injection of bortezomib, the patient presented an isolated chest and face papulonodular erythema without prurit that resolved spontaneously in 2 weeks. A skin localization of his lymphoma was first suspected as the patient had no history of allergy and skin diseases. Two days after the seventh administration of bortezomib, the patient developed the same skin reaction that also resolved within 2 weeks. A cutaneous biopsy of the lesion evidenced a diffuse mononucleous infiltrate including histiocytes and few T-cells and showed no histological and immunological arguments for a skin involment of his lymphoma. All concomitant medication (sulfamethoxazole + trimethoprime, folic acid and quinine) administered with bortezomib that could be potentially involved in this skin reaction were withdrawn. One day after the tenth injection of bortezomib, the chest and face papulonodular erythema occurred again with the same intensity. A biopsy specimen of the skin lesion revealed a dermal-hypodermal perivascular infiltrate of mononuclear cells with T-cell and histiocytes. Thus, it was decided to stop permanently bortezomib as drug-related hypersensitivity was strongly suspected. Patient recovered without sequelae of the last skin reaction within 1 month. Later on, the patient was tested at the allergologic department. At 48 hours, patch tests with bortezomib were negative but the intradermal test with bortezomib returned positive (dilution 1:10).
Conclusion: A few cases of bortezomib-induced skin reactions are described on the literature. Our patient presented a skin reaction that recurred with subsequent cycles of bortezomib and disappeared after discontinuing the drug. These facts strongly suggest a bortezomib-related skin reaction. The allergic tests confirmed that the toxic skin reaction was a delayed-type hypersensitivity to bortezomib. This case reports for the first time an allergy to bortezomib.

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Intravenous immunoglobulins-induced eczematous eruption: a long-term-follow-up study
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Introduction: High-dose intravenous immunoglobulins have emerged as an important therapy for various diseases. Vesicular eczematous eruption has recently been described as an adverse event of this treatment. However, little is known about patients' characteristics, administration regimens and long-term outcomes.
Methods: We retrospectively examined from medical charts, a series of eight consecutive patients which had been notified to the Regional Pharmacovigilance Center for an eczematous skin reaction after intravenous immunoglobulins infusion. We also performed an extensive search from Medline to review the reported cases.
Results: Among the eight patients of our series, there were seven men and one woman. Mean age was 52.5. Only one patient had a history of allergy. Seven patients were treated with intravenous immunoglobulins for neurological disease. The infusion rate was 0.4 g/kg/day during 5 days for five patients and 1 g/kg/day during 2 days for the remaining three patients. Six out of eight patients experienced skin eruption after the first infusion of intravenous immunoglobulins. Median delay from the onset of immunoglobulins infusion to eruption was 9.5 days (range: 3–21). Eruption was mostly localized to palms (n = 4) and soles (n = 3) and was pruriginous in seven patients. All patients improved, either spontaneously or with steroid treatment, with a mean delay of 21 days (range: 10–30). Immunoglobulins were stopped in three patients. Rash recurred in four of five patients in which immunoglobulins were readministered, with a more widespread eruption in all. However, eczematous eruption was attenuated from the third administration. Eruption did not relapse in three patients when immunoglobulins preparation was switched for another one. At the end of follow-up, no patient had sequelae, except one which complained of persistent pruritous.
Skin side-effect of immunoglobulin therapy has been reported to occur in up to 6% of treated patients. We identified from Medline 29 reported cases of immunoglobulins induced eczematous eruption. Seventy-five per cent were treated for neurological disease. As in our series, eruption concerned mostly palms (85%) and soles (35%) and was usually pruriginous (81%). Patients were commonly treated with topical (45%) or systemic (14%) corticosteroids, although others improved without treatment. Treatment was re-introduced in 12 patients and 11 relapsed (six of whom with a more widespread eczematous eruption). Switching the type of preparation was successful in two cases, without eruption recurrence.
Conclusion: Eczematous eruption due to infusion of immunoglobulins is a mostly benign side-effect. Treatment withdrawal is not required if there is a clinical benefit. Changing the type of preparation may allow reintroduction without rash recurrence.

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Severe cardiac events associated with the discontinuation of nadolol treatment in three patients
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Introduction: Beta-blockers are used in various dysrythmias influenced by adrenergic tone. These drugs are thus considered as the mainstay of therapy in most forms of long QT syndrome (LQTS). Among these drugs, nadolol has been presented by some authors as particularly useful to prevent ventricular arrhythmias or sudden death especially when precipitated by exercise or emotion.
Methods: Recently, fabrication problems by the manufactures of nadolol led to a transitory discontinuation of the drug distribution. Three cases of severe arrhythmias in patients previously treated by this drug occurred in this setting. We discuss in this presentation some questions raised by these case reports.
Results:
Case 1: A 13-year-old boy presenting a congenital LQTS linked to a KCNJ2 mutation (with exercise-induced ventricular tachycardia) was treated with an implanted automatic defibrillator and by nadolol 80 mg/day. Because of the lack of availability of the drug, bisoprolol had been given. A week later, during a sport meeting, the boy presented with an electrical storm and a cardiac arrest necessitating a 20 min resuscitation manoeuvres but leading to major neurological alterations and death some days later.
Case 2: A 21-year-old man was known as presenting a Jervell and Lange–Nielsen syndrome (congenital LQTS associated with deafness). He was treated by nadolol (100 mg/day). The lacking drug was changed to atenolol (100 mg/day). Some days after, during a football meeting, he presented two syncopal episodes. A cardiac arrest occurred. Resuscitation manoeuvres and prolonged reanimation were necessary. Following outcome was favourable. There were no sequelae.
Case 3: A 31-year-old received nadolol (40 mg/day) since November 2005 for ventricular tachycardias and dilated cardiomyopathy. The drug was substituted by bisoprolol (10 mg/day). Two weeks later the patient presented with syncope related to a recurrence of ventricular tachycardia. In these three cases, severe ventricular arrhythmias occurred soon after the replacement by other beta-blockers of a prolonged treatment by nadolol. This led to question the particular profile of action of this beta-blocking drug in ventricular arrhythmias in the setting of channelopathies or cardiomyopathy.

Conclusion: The relatively short delay of onset of life threatening arrhythmias twice during marked exercise suggests that beta-blockade induced by nadolol was indeed effective to prevent adrenergic mediated dysrhythmias. A greatest efficacy than other beta-blockers is by that way suggested. Thus, the replacement from nadolol to another beta-blocked may be deleterious in selected patients.

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Ischemic colitis and neuroleptics – a retrospective study in a psychiatric hospital and a new physiopathologic hypothesis

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Introduction: Ischemic colitis is a very serious side-effect of neuroleptics. Pathogeny of neuroleptic toxicity is not yet determined. The aim of the study was to have a better knowledge of this side-effect in order to improve management and to evaluate ischemic colitis frequency.

Methods: From 1992 to 2003, patients with ischemic colitis had been included in a retrospective study in a psychiatric hospital. We collected data on patients' characteristics, diagnosis and risk factors of colitis, medicines, management and evolution of ischemic colitis. We attributed a gravity level to ischemic colitis and compared it to the number of neuroleptics and anticholinergic treatments.

Results: Eleven patients had been included. Eight cases underwent surgery. Two of them had fatal outcome. Clinical symptoms were digestive and not specific: diarrhea (7), abdominal pain (6), vomiting (6) and occlusive syndrome (2). Diagnosis was based on clinical symptoms (11), coloscopy (5) and histology (8). Infectious and inflammatory colitis had been eliminated. The average age was 48-years-old, they were suffering from serious mental disorders and were in psychiatric hospital for a long time (average: 12 yrs). History of digestive disease was the most frequent risk factor (6). Ten patients received at least one neuroleptic, eight of them with heavy dosages. Furthermore, we estimated the ischemic colitis frequency in our hospital about 1/2400 p. years. Vomiting was often found as an alarm symptom of colitis. Recognition of prodromal symptoms should facilitate management to reduce morbidity and mortality. Special care to patient undergoing large dosage of neuroleptics (and anticholinergic correctors) or suffering from constipation is required by physicians. Prevention may be based on constipation management and psychiatric treatment periodic assessment. A correlation is observed between number of neuroleptics and anticholinergic treatments and colitis severity, suggesting a dose-dependant toxicity. Anticholinergic potential of psychiatric treatments is usually involved in this side-effect. We suggest another mechanism. Dopamine produces forearm vasodilatation following alpha-adrenoceptor blockade by an action on vascular dopamine (DA1) receptors in man. Inhibition of mesenteric-DA1 receptors by neuroleptics could be responsible for the non-dilatation of preconstricted mesenteric arteries and lead to mucosis ischemia. Finally, compared with the general population, the estimated frequency is clearly higher in this study (41.6 vs. 4.5 to 9.9/100 000 p. years).

Conclusion: Ischemic colitis is a rare but very serious adverse effect of neuroleptics, requiring attention and information to prevent fatal evolution. Furthermore, vascular DA1-receptors antagonism hypothesis have to be explored.

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Thiocolchicoside in the first trimester of pregnancy, a french collaborative study

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Introduction: Thiocolchicoside is a muscle relaxant which is widely used in France to treat painful contractions. High doses of this drug is reported to be teratogenic and foetotoxic in animals as stated in the french SPC which is thus alarming. However, there are no published human data to assess the safety of this drug during pregnancy. The aim of the present work is to study available French data collected by regional centres of pharmacovigilance (CRPV).

Methods: Prospective cases of thiocolchicoside exposure during the first trimester of pregnancy were extracted from TERAPPEL, the French database used by some CRPV's to share cases of women exposed to xenobiotics during pregnancy.

Results: One hundred and sixteen cases were recorded in the database. Among them 74 had documented evolution. They concern women between 20 and 43 years old. For eight cases, thiocolchicoside was the only drug taken. More often NSAIDs ($n = 60$), analgesics ($n = 35$) or tetrazepam ($n = 17$) were associated with this drug. Pregnancy outcomes included 57 live births, 10 miscarriages, six voluntary abortions and one medical abortion (for maternal reason). Among live births, no major malformation and one minor malformation (hip luxation) were observed.

Conclusion: To our knowledge this is the first report study about thiocolchicoside exposure during human pregnancy. In this study the overall incidence of congenital abnormalities was found very low but the sample size was not sufficient to discard a teratogenic effect of thiocolchicoside. It is worth noticing that these reassuring data do not confirm the animal studies.

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Macrolide and cephalosporin antibiotic-induced eosinophilic myocarditis

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Introduction: Macrolides are known to have relatively few side effects. We report an eosinophilic myocarditis (EM) in a patient following intake of the macrolide antibiotic roxithromycin, spyramycin and cefepime, fourth-generation cephalosporine antibacterial. Myocarditis is rare and eosinophilic myocarditis is rarer, eosinophilic myocarditis may be related to multidrug therapy.

Methods: We describe a man presenting a rare case of eosinophilic myocarditis after exposure to macrolide and cephalosporine antibacterials.

Results: We report a case of a 51-year-old man with medical history of idiopathic hypogammaglobulinemia, with T4 deficiency but without human immunodeficiency virus and CMV positive without clinical symptom. He had a history of hepatic cytolysis in June 2006, his liver was infiltrated with granuloma (antineutrophil cytoplasmic antibodies were negative). He is treated by polyvalent immunoglobulin, fluticasone, salmeterol and salbutamol. This patient present no heart disease. On the 2/11/06, he suffered from a splenomegaly which lead to a splenectomia. The 9/11/06, he was admitted to hospital for an acute respiratory distress on an interstitial pneumopathy and was treated by spiramycin (10–13/11/2006) and cefepime (10–13/11/2006) and then by cefepime (13–20/11/2006) and roxithromycin (13–20/11/2006). He developed an eosinophilia on the 13/11/2006 (10.1%) and an eosinophilic myocarditis on the 17/11/2006 (23%). The diagnosis of myocarditis was confirmed histologically. The differential diagnosis of Churg–Strauss syndrome is discussed but autoimmune diagnostic test was negative for ANCA. Treatment with steroid therapy produced a dramatic improvement in symptoms and eosinophilia. The incidence of EM is low but probably underestimated. EM can be observed in any case involving polynuclear eosinophils (PE) and has been described in cases of drug-induced allergy like in our case, in Churg and Strauss syndrome, and in essential eosinophilic syndrome. The synthesis of the anatomo-clinical and experimental data suggests a myocardial aggression by cytotoxic effects of granular protein component released during activation of polynuclear eosinophils.

Conclusion: The heart is a prime target for PE toxicity, some authors suggested that EM result from the action of PE on endothelial cells for EM. The diagnosis was established on the basis of clinical criterias and laboratory investigations. This case suggests that EM may be a complication of roxithromycin, spiramycin or cefepim, even though it is probably rare.

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Is sirolimus-induced proteinuria reversible by angiotensin II receptor blockade?

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Introduction: Sirolimus is a new and potent immunosuppressive agent approved for maintenance therapy after kidney transplantation. Recently, increased proteinuria has been recognized as an important side-effect of sirolimus.

Methods: Case report.

Results: Two renal transplant patients received sirolimus as a first-line immunosuppressive therapy associated with mycophenolate mofetil and corticotherapy. The first male patient, aged 49 years, was grafted in January 2005 because of past medical history of polycystic kidney disease. Fifteen days after renal transplantation, all biologic parameters returned to normal levels. A 2-month post-transplant examination, an isolated proteinuria (2.2 g/24 h) without hematuria was observed. The blood creatinine level was ranging from 120 to 140 μmol/l, no hypertension was diagnosed and sirolimus blood level remained in the therapeutic range (10–15 ng/mL).The renal biopsy performed to investigate the proteinuria showed no signs of graft rejection but evidenced mild glomerular damage. It was decided to maintain the sirolimus therapy and to introduce an angiotensin II receptor blocker. A few days later, proteinuria gradually improved and no episodes of proteinuria occur anymore.The second patient, a 44-year-old man was successfully grafted in April 2005 because of history of bilateral hydronephrosis. After one month of sirolimus medication, the patient presented an isolated severe proteinuria reaching 4.4 g/24 h. Blood creatinine level was normal and sirolimus blood level was within therapeutic range. The histological findings from the renal biopsy evidenced both glomerular and tubular damages but no signs of acute graft rejection. As the previous case described, sirolimus was maintained and an angiotensin II receptor blocker was initiated. One month later, the urinary protein excretion returned to normal values.

Conclusion: From data available in the literature, a cause-and-effect relationship between proteinuria and the exposure to sirolimus seems now well-established. The renal biopsies of these two patients evidenced glomerular damages that suggest a direct toxicity of sirolimus. Furthermore, sirolimus-related proteinuria can interestingly be reversed by angiotensin II receptor blocker. This observation underlines a possible nephroprotection of angiotensin II type 1 receptor blockers in sirolimus-treated renal transplant patients. Therefore, these former patients should be carefully monitored in order to detect any adverse effects on renal function.

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Dextropropoxyphene withdrawal from the list of drugs available in a French University Hospital: consequences on consumption of analgesic drugs

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Introduction: Dextropropoxyphene is a widely used weak opioïd analgesic marketed in France in association with paracetamol. Considering its potentially serious adverse drug reactions, the Toulouse Hospital Drug Committee first emitted several alerts towards prescribers (2002, 2003, 2004) and then decided to exclude dextropropoxyphene from the list of drugs available for inpatients from the 1st June 2005. The aim of our study was to compare the use of analgesics inside Toulouse University Hospital before and after dextropropoxyphene withdrawal.

Methods: We investigated consumption of analgesics from the three steps of World Health Organization classification over a 7-year period (2000–2006) and compared 2006 and 2004 data. Results were expressed in defined daily doses for 1000 hospitalisation-days (DDD/1000 D).

Results: From 2000 until 2006, the overall analgesics use increased by 10% (from 706 DDD/1000 D to 779 DDD/1000 D). In 2004, consumption was 755 DDD/1000 D. Drugs from steps 1, 2 and 3 represented 56%, 35% and 9%, respectively. Dextropropoxyphene in association with paracetamol was the second most used analgesic after paracetamol. Although decreasing since 2002, its consumption was 139 DDD/1000 D i.e. 18% of total analgesic consumption and 52% out of step 2. In 2006, after dextropropoxyphene withdrawal, the use of tramadol increased in comparison with 2004. This increase involved tramadol associated with paracetamol (51 DDD/1000 D versus 5 DDD/1000 D), oral prolonged-release form (47 DDD/1000 D versus 29 DDD/1000 D) and injectable form (15 DDD/1000 D versus 8 DDD/1000 D). We observed no transfer to codeine in association with paracetamol. The overall consumption of step 2 analgesics decreased by 22%

(206 DDD/1000 D versus 265 DDD/1000 D). At the same time, step 1 analgesic consumption increased by 19% (507 DDD/1000 D versus 425 DDD/1000 D). This concerned mainly oral or rectal paracetamol (348 DDD/1000 D versus 285 DDD/1000 D). Step 3 analgesics remained stable.
Conclusion: Our study shows that withdrawal of dextropropoxyphene from the list of drugs available for inpatients did not lead to a complete transfer to other step 2 analgesics. We can assume a partial transfer to step 1 analgesics. Nevertheless, there was no complain about the quality and efficiency of pain management. These results are consistent with a possible misuse of dextropropoxyphene, being considered as a common first-line analgesic, and/or an overconsumption linked to abuse by some inpatients. This study emphasizes the role of an hospital Drug Committee in supporting rational use of drugs and preventing adverse drug reactions.

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Toxic epidermal necrolysis associated to leflunomide treatment
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Introduction: Leflunomide (ARAVA®) is a new immunomodulatory and anti-inflammatory agent which is effective in the treatment of rheumatoid arthritis. It exerts antiproliferative effects on activated lymphocytes through inhibition of *de novo* pyrimidine synthesis. It is generally well tolerated; however, adverse drug effects often occur, including allergic cutaneous reaction and are moderate. In our study, we reported a patient with toxic epidermal necrolysis (TEN) induced by leflunomide.
Methods: We report a case of a TEN induced by leflunomide notified to the Tunisian National Centre of Pharmacovigilance and validated by Begaud's method.
Results: A 36-year-old woman, with a history of miscarriages, treated initially by methotrexate for rheumatoid arthritis (April 2005). On December 23, 2005 (day 1) methotrexate was stopped and leflunomide was introduced. On day 13, she made generalized convulsion and cerebral tomography scan was normal. At the same day, leflunamide was stopped. Two weeks later she presented maculo papulosis erythema of the face, the neck and the dorsum associated to pustules, chilitis and conjunctivitis. On day 38, there was extension of the cutaneous lesions to the abdomen and limbs with palmoplantar eruption. The next day, in front of the aggravation of the symptomatology and cutaneous detachment, she was hospitalized in intensive care unit. The biologic tests showed a cytolytic hepatitis (seven normal values), pancytopenia, and a normal renal function. The cutaneous biopsy confirmed TEN diagnosis. The course was marked by cutaneous detachment in shreds and nails detachment. The evolution of cutaneous lesions was favourable, but the patient kept blindness.
Conclusion: The responsibility of leflunomide in genesis of TEN was retained in front of a compatible delay of iatrogenic origin and a negative etiologic investigation. In the literature, principal adverse effects of leflunomide therapy include gastrointestinal symptoms, elevated liver enzymes, hypertension, headache, alopecia, and allergic cutaneous reaction. Allergic cutaneous reactions are mostly mild to moderate. Other skin reactions have also been reported, including vasculitis, lichenoid drug eruption, lupus erythematosus, and Stevens–Johnson syndrome. TEN have been exceptionally reported.

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Adverse reactions to valproic acid
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Introduction: Valproic acid (VA) is an anticonvulsant drug widely used in partial or generalized seizure and for bipolar disorders. Its most side effects are generally benign as digestive disorders, somnolence, hyper-ammonemia and cutaneous reactions. Serious side effects are rare as pancratitis, hepatitis and serious cutaneous reactions.The aim of our study was to evaluate type of side effects.
Methods: We performed a retrospective study witch concerned all suspected cases of adverse reactions due to VA. These cases were notified at the Tunisian National Centre of Pharmacovigilance between December 1990 and December 2005 and validated according to the French method of imputability of Begaud and all.
Results: Twenty-six cases were retained. Our patient group was formed of 16 women and 10 men. The middle age was 19.5 years (min/max 2 months/52 years). VA was used for epilepsy in 20 cases, for seizure in three cases, for manic-depressive psychosis in two cases and for cerebral malformation in one case.VA was used in monotherapy in 16 cases and in association with other drugs in 10 cases.The delay between onset of the event and VA administration varied from 12 hours to 6 years. Events associated to VA use were: benign cutaneous effects in 14 cases (cutaneous eruption: 10 cases, fall of hair: three cases and pruritus: one case), sever cutaneous reactions in one case (toxic epidermal necrosis), digestive effects in 11 cases (cytolytic hepatitis: six cases, increase of alkaline phosphatase levels: three cases, pancreatitis: one case and epigastralgia: one case) and tremor in one case.
Conclusion: In our study cutaneous reactions were seen in more than half cases (15/26) in which one case of toxic epidermal necrolysis. Digestive disorders were observed in 11/26. In the literature, the most frequently reported adverse effects associated with VA are gastrointestinal disturbances. Pancreatitis and bullous dermatitis were rarely described.

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Survey of danaparoïd use in an university hospital
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Introduction: Danaparoïd sodium is an alternative anticoagulant in patients who develop heparin-induced thrombocytopenia. This prospective study was performed to determine the number of patients who were delivered danaparoïd after an heparin-induced thrombopenia between 1 January and 31 May 2006 during their hospitalization at Reims university hospital.
Methods: After identification of the patients having received danaparoïd at central pharmacy, a retrospective analysis was carried out in concerned units and in the hematology laboratory between 1 January and 31 May 2006.
Results: Thirty-six patients were treated by danaparoïd, 45% in cardiovascular medicine or surgery units. The indication of danaparoïd was correlated with at least one positive test of heparin-induced thrombocytopenia in eight patients: two patients with both positive platelet and anti-FP4/heparin antibodies test, three patients with positive platelet and two positive anti-FP4/heparin antibodies test. The other indications were a previous heparin-induced thrombopenia or heparin cutaneous allergy in the past for four patients, cutaneous allergy to heparin during his hospitalization for one patient. The information was incomplete in seven cases. For 13 patients the diagnosis of heparin-induced thrombopenia wasn't confirmed. Three prescription errors were detected (off label use). None of the eight suspected cases were notified to the Regional Pharmacovigilance Center.
Conclusion: Heparin-induced thrombocytopenia remains a serious iatrogenic effect. His early diagnosis is important to avoid thromboembolic reactions and to prescribe an alternative treatment to some patients. In practice, diagnosis remains difficult among surgery patients, who require a hypocoagulation despite an often low platelet level. In our survey, danaparoïd wasn't always justified, whereas it is not exempted of adverse effects and quite expansive. A better diagnosis and a rigorous follow-up of deliveries could improve the prescriptions.

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Development and validation of a new method of determination of Itraconazole in human plasma by high-performance liquid chromatography
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Introduction: Itraconazole intra- and inter-patient variability in the pharmacokinetics parameters, and the fact that it is impossible to predict steady-state plasma concentrations from the initial dosage are major factors obscuring any clear relationship between dose and plasma concentrations and clinical efficacy. Thus plasma concentrations should be regularly monitored to ensure sufficient drug exposure for antifungal activity. Several assays were used to quantify Itraconazole in plasma with an etalonnage curve using at less five standards.
The aim of our study was to optimise and validate a new method (simple and sheaper) to dosing Itraconazole in human plasma by high performance liquid chromatography (HPLC), using an internal standard.
Methods: A HPLC was used to quantify Itraconazole in human plasma.
Results: We have selected initial chromatographic conditions: separation was achieved with a reversed-phase C_{18} (250 mm) and UV detection (λ = 263 nm). The mobile phase consisted of ammonium acetate adjusted to pH = 8 and acetonitrile (46/54, V/V) at a flow rate of 1 mL/min. We have tested many internal standards (Thiopental, Théophylline, Carbamazeipine, Butalbarbital, Indométacine, and lidocaïne) and we opted for Lidocaïne, because of its good coefficient of resolution R_s = 2 (between Itraconazole and Lidocaïne). Retention time of Itraconazole and Lidocaïne were 12.8 and 4.5 minutes respectively. The method involved liquid-phase extraction of Itraconazole and Lidocaïne based on methanolic extraction of these compounds from plasma.
The curve was linear from 50 to 3200 ng/mL with a correlation coefficient r = 0.9999. The inter- and intra-assay precision values were measured by the per cent coefficient of variation which are 3.408 and 2.146%. The limits of quantification and detection levels were 0.065 and 0.021 µg/mL respectively. The calibration curve regression (y = ax + b) suggest a perfect correlation between (pic height ratio) and theoric concentration.
Conclusion: Our new analytical method using an internal standard allowed us to using only one standard instead of five standards. This method is simple, and robust enough to quantify Itraconazole in blood. This procedure permit therapeutic drug monitoring, susceptible to predict efficacy and toxicity in patients receiving Itraconazole.

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Observational study about the prevalence of bleeding and thrombosis in patients with coronary stents and clopidogrel-aspirin combination therapy
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Introduction: Drug eluting stents are associated with a decrease in restenosis, however they also require prolonged dual antiplatelet therapy to avoid thrombotic complications. Currently, lack of international guidelines is leading to empirical behaviour towards the length and dosage of this dual therapy. The primary objective of this observational study was to assess the impact of real life management on the prevalence of dual antiplatelet related adverse drug reaction (bleeding and thrombosis) in patients with coronary stents. The secondary objectives was to identify risk factors associated with these ADR.
Methods: We conducted an observational retrospective study analysing the outcome of 1180 patients with coronary disease admitted for stent placement at the University Hospital of Toulouse between January 2004 and 2005. We collected information about the clinical, biological and angiographic status of each patient. We identified severe ADR and all bleeding and thrombosis cases occurring during one year follow up. We calculated the incidence of ADRs and logistic regression analysis identified factors associated with the occurrence of bleeding or thrombosis.
Results: The annual incidence of haemostasis-related ADR was of 22.7%. Most of them occurred within the first 15 days (79%). Bleeding occurred in 19.2% of the patients (among them 2.54% were severe) and 4.8% of patients had intra stent thrombosis. Mortality rate was of 1.9% bivariate analysis identified combination of beta blocker, aspirin, ACE inhibitors and statin as a protective factor for bleeding while alcohol abuse (>30 g/days) increases the thrombotic risk. In multivariate analysis anaemia [*OR* = 2.2, (IC95: 1.5–3.2)] arterial hypertension [*OR* = 1.7 (1.1; 2.4)] and patients who received a stent for the first time [*OR* = 2.3 (1.4; 3.8)] were conditions significantly associated with bleeding. Risk factors for intra stent thrombosis were as expected: early discontinuation of dual antiplatelet therapy, increased number of red cells and platelets but also occurrence of a previous stroke.
Conclusion: This observational study shows that independently of their severity, bleeding and thrombosis are relatively frequent in patient receiving dual antiplatelet therapy as one out of five patients will experience one of this ADR. Early identification of risk factors could decrease the risk of these ADR but this need to be confirmed in prospective randomized trial.

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Valvular heart disease in patients with parkinson's disease treated with pergolide

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Introduction: Restrictive valvulopathy has been reported with pergolide, an ergot- derived dopamine agonist indicated in the treatment of Parkinson's disease (PD). Few data are available to estimate the incidence, severity and reversibility of this induced valvular disease.

Methods: Evaluation of frequency, grading of valvular regurgitation and relationship to pergolide cumulative dose as well as reversibility after drug cessation. The transthoracic echocardiography findings were analysed in 30 patients and compared with those obtained in control patients (patients without PD referred for echocardiography but without known valvulopathy) and matched for age and sex. In 10 PD patients with restrictive valvulopathy, a new echocardiography was performed after a mean pergolide interruption of 14 months.

Results: Thirty PD patients treated with pergolide were compared with 30 controls (same sex ratio 15/15 and age 67.8 years ± 10 vs. 67.2 ± 9.7 NS). A pattern of valvular restrictive regurgitation was observed in 13/30 patients taking pergolide (43%). Two patients had heart failure symptoms. Compared to controls, aortic as well as mitral regurgitation appeared to be much more frequently observed in PD patients with an odds ratio of respectively 3.1 (95 IC 1.1–8.8) and 10.7 (95 IC 2.1–53). The increase in frequency of tricuspid regurgitation was not significant. The number of affected valves and the sum of regurgitation grading were significantly higher in the pergolide group. No correlation could be found between cumulative dose of pergolide and severity of regurgitation. In 10 out of the 13 patients presenting a valvulopathy associated with pergolide, the drug was withdrawn. In six of these patients, regurgitation grades were found to be lower 10–18 months after drug withdrawal (no change in the four other cases). The two patients with heart failure returned to nearly normal examination.

Conclusion: This study confirms the high frequency of valve regurgitation in PD patients treated with pergolide [33% in the Van Camp's study (1)] and documents that a significant improvement may be observed when pergolide is stopped.

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Knowledges of pharmacological bases of antimalarial drugs artemisinin based combination therapy: study close to Ivorian prescribers

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Introduction: The new policy of Fight against malaria program of health minister recommended the use of artemisinin-based combination therapy (ACT) for *P falciparum* malaria treatment in order to avoid resistance. The clinical pharmacology service carried out for the public health insurance (state employee general insurance of Cote d'Ivoire 'MUGEF-CI') a pre-test on pharmacological bases of ACT.

Methods: A total of 172 prescribers divided up seven groups were participated to the study. The physicians came from different steps of national sanitation system. Questionnaire was performed on five themes: definition, objectives of treatment, pharmacokinetic base and name and International Common Denomination of available ACT in Cote d'Ivoire. The questionnaire proposed 16 items with 12 valid items.

Results: The average of appreciation out of 20 was 11.62 ± 0.725. 60% of good responses were noted. 89.8% of physicians defined correctly ACT except for Sulfadoxine-pyrimethamine (52.1%). Concerning the objectives, the rate of just responses was 63.1% with variations: amelioration of efficacy (79.4%) delay of pharmacoresistance emergence (55.6%) and synergic action (54.2%). The principle of ACT based on half life was known by 79.7% of participants.

The ACT recommended in first intention were known by 78.7% of physicians and those of second line by 46.8%. 2.3% know none ACT and 4.5% could well associate selling name to corresponding International Common Denominations.

Conclusion: Pharmacological bases of ACT were unknown by many health professionals. This situation could induce misuse or abuse and failure of national policy about fight against malaria.

Keywords: Knowledges – pharmacology – antimalarial drugs -combination therapy.

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Heterogeneity of the information for dose adjustment in renal impairment according to the French drug compendium specifications

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Introduction: The use of drugs in patients with renal impairment is based on dose recommendation from the French summary of product characteristics (SPC) most easily accessible through the 'Vidal'. This reference information is however heterogeneous and at times misleading. It is generally admitted that serum creatinine concentration (sCR) is only a rough guide for dose adjustment in patients with renal impairment. Creatinine clearance (CRCL) is a better estimator. Under the usual clinical conditions, this clearance can only be evaluated through approximations such as the Cockcroft-Gault or more recently the MDRD formulas. The objective was to review dose adjustment in renal insufficiency proposed in the SPCs of some drugs eliminated by the kidney.

Methods: The inconsistencies of some drug SPCs have been identified and reported hereafter from the Vidal.

Results: The following incoherencies were found in the SPC of various drugs when administration to patients with renal impairment is considered:

Antiviral drugs: the magnitude of the dose reduction in renal insufficiency is less with valacyclovir (one half of normal dose) than with acyclovir (one fourth of normal dose). Following this schedule, in a patient undergoing haemodialysis and given valacyclovir, induced an acyclovir accumulation episode with confusion and hallucinations.

Aminoglycosides: both sCR and CRCL are proposed for dose adaptation of aminoglycosides. Using these two variables, we calculated dose adjustment in patients in an intensive care unit given gentamicin. The dose evaluated from sCR was 216 ± 97 mg/day and from CRCL was 124 ± 71 mg/day.

Beta-blockers, converting enzyme inhibitors, oral antidiabetics: the clearance limits proposed for dose adaptation are different from one drug to the other. In the SPCs of oxprenolol and metoprolol, information on dose adjustment in renal insufficiency and in the elderly is conflicting. Metformine adaptation is based solely on sCR.

Statins: the magnitude of dose adjustment according to the various grades of renal insufficiency is not clearly proposed.

Conclusion: These information discrepancies are confusing and could at times induce inefficiency or toxicity as a consequence of an inappropriate dose schedule. The degrees of renal insufficiency used as guideline for the determination of the initial dose of a treatment should be the same for all drugs. A harmonization of information on drug dose adjustment is necessary. Taking all these points into account is a huge task for a future group encompassing pharmacologists, nephrologists and geriatricians.

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Bullous drug eruption: 24 cases reported in Sfax pharmacovigilance unit

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Introduction: Bullous drug eruption is a rare and severe adverse drug reaction. We report 24 cases during 7 years. The causality assessment was treated by two methods.

Methods: A retrospective study was carried out in pharmacovigilance unit of Sfax from January 1999 to December 2005. Two methods were proposed to assess the causal relationship between a drug treatment and the occurrence of this adverse event: French imputation method (MIF) and new probability method proposed by Begaud et al (using a logistic model: MIL).

Results: Twenty-four bullous drug eruptions were reported during 7 years (3.4 cases/year). They represent 7.6% of all cases of drug adverse event and 13.7% of all cases of cutaneous drug reactions. During this period, we have reported nine erythema multiforme, nine Stevens Johnson syndrome, three toxic epidermal necrolysis and three fixed drug eruption. Analgesic-antipyretics (19.23%), anticonvulsivants (19.23%), nonsteroidal anti-inflammatory drugs: NSAIDs (15.38%), sulfonamide antibiotics (15.38%), other antibiotics (11.53%), diuretics (11.53%), allopurinol (3.84%) and anti histaminic (anti H2) (3.84%) were the main responsibles of this adverse event. In 2 cases, the score of imputability was (doubtful) using MIF and the probability was ranged between 73 and 88% using MIL. In 15 cases, the score of imputability was (plausible) by MIF and the probability was ranged between 73% and 92% by MIL. In eight cases, the score of imputability was (likely) by MIF and the probability was ranged between 73% and 95% by MIL. Finally, in one case, the score of imputability was (very likely) by MIF corresponding to a probability of 95% using MIL.

The originality of the new method stems from the use of a logistic model which combine notoriety and semiology and identifies very well the criteria of time to onset.

Conclusion: Practitioners should be informed of this risk and collaborate with pharmacovigilance center. The new method of imputability seems to be interesting but should be validated by a larger sample.

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Bullous drug eruptions and autoimmunity diseases

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Introduction: Bullous drug eruption is a rare and severe adverse drug reaction. The association with autoimmunity disease seems not to be fortuitous. We report four cases of bullous drug eruptions associated with autoimmunity diseases.

Methods: A retrospective study was carried out reporting all cases of bullous drug eruptions associated with autoimmunity diseases in pharmacovigilance unit of Sfax from January 1999 to December 2005.

Results: Twenty-four cases of bullous drug eruption were notified during 7 years. Among them, four patients (16.66%) presented autoimmunity diseases: one rheumatoid arthritis, two ulcerative colitis and one nephrotic syndrome. Average age was 52.25 years; the male/female was one. This association reinforces the immunology hypothesis.

Conclusion: Bullous drug eruptions are severe adverse drug reactions. Practitioners should be informed of this risk especially in patients who had autoimmunity disease.

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Valproate-induced encephalopathy triggered by psychotropic drugs association

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Introduction: There have been several reported cases of valproate-induced encephalopathy with hyperammonemia. The mechanisms by which valproate can be associated with encephalopathy have not been completely elucidated, but elevated serum levels and hyperammonemia might be responsible for the toxic effects on the brain. However, valproate associated with psychotropic drugs may conduce to severe encephalopathy in the absence of elevated serum ammonia level.

Methods: Case report: A 36–year-old woman, hospitalized for a second brief psychotic episode, was first treated with diazepam 30 mg/day. Divalproaex sodium 1000 mg/day was added 1 week later. Zopiclone was occasionally administered. Over the following 3 days, divalproex sodium was increased to 1500 mg/day and olanzapine 10 mg/day was added. As this latest combination was started on day 4, she slowly became comatose in few days. Electrolytes, liver and renal function tests were normal. Her serum valproate level remained slightly elevated at 120 mg/L (normal: 50–100). The ammonia level was 26 µmol/L (normal: 11–48). Although her serum valproate levels dropped to 77 mg/L, she was deeply comatose in a non-reactive state some hours later. Twenty-four hours later all treatments were discontinuated, she had completely recovered.

Results: This case emphasizes that valproate can induce severe encephalopathy in conditions of drugs association by a different mechanism from toxic ammonia accumulation.

The hypothesis of metabolism interactions is unlikely because hepatic metabolism of these drugs seems to be different.
Although it is possible that pharmacokinetic interactions between valproate and psychoactive compounds have contribute to encephalopathy by addition of their effect on the brain, the hypothesis of an overdosage with free drugs seems to be more plausible. Indeed, valproate, diazepam and olanzapine are highly bound to plasma proteins (81–90%, 95–98%, 93% respectively) with competition between drugs for binding. It is known that the free fraction of valproate is non linear and increases rapidly with the dose due to saturable plasma protein binding. Potential plasma high concentrations of free fraction might induce toxic effect. Serum valproate levels may have misled practitioners because they reflect total concentrations and may appear to be normal whereas free concentrations may be elevated.
Conclusion: The decreased serum protein binding of valproate during psychotropic association and addition of pharmacodynamic effects might result in severe encephalopathy. Dose reduction should be considered in patients taking drugs which are highly bound to proteins. This emphasizes the potential usefulness of the free fraction determination in therapeutic drug monitoring.

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Automatised detection of adverse drug reactions in discharge summaries: a feasibility study

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Introduction: Various methods of adverse drug reactions detection exist: spontaneous reporting is by far not exhaustive, chart review is very time costly. Analysis of electronic discharge summaries is a promising tool. But it still represents an amount of work (8000/month in the Grenoble teaching hospital). This study tries to answer the following question: is it possible to list keywords to detect in a fast and efficient way adverse drug reactions in computerised discharge summaries, and to use this method on a regular basis?
Methods: The study population was inpatients who went out in February 2006 for the first sample, and between the 1 and the 7 of March for the second sample whom discharge summary was accessible via a local software developed for the medical information unit for coding purposes. On the first sample, exhaustive human reading of discharge summaries allowed to detect adverse drug reactions and by the way, to establish a first large keywords list. These keywords were submitted to automatised detection with Acrobat Reader® on the first sample to determine their false positive rate. A more powerful second keywords list was established to decrease false positive rates. On the second sample, automatised detection with the second keyword list was performed and compared with exhaustive human reading, for final calculation of sensitivity, specificity, negative and positive predictive values.
Results: On the first sample, 2045 discharge summaries were scrutinised. 275 descriptions of adverse drug reactions were retrieved corresponding to 265 cases. 131 adverse drug reactions could be characterised by 44 keywords. 11 keywords with a high false positive rate were cancelled or transformed, giving a second list of 37 keywords. 46% of adverse drug reactions were detected, sensivity: 44.4%, specificity: 91.3%, positive predictive value: 44%, negative predictive value: 91.4%. On the second sample, 43.6% of adverse drug reactions were detected, sensitivity: 32%, specificity: 91.6%, positive predictive value: 43.6%, negative predictive value: 84.9%. Age, sex ratio, nature of the reaction, sensitivity, specificity, negative and positive predictive values were statistically identical in the two samples.
Conclusion: This method does not allow an exhaustive detection, but increases signal potency, and is timesaving, resulting in a very good output. The keywords list is adaptable to various studies subjects. It could be optimised by using boolean search softwares.

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Drug induced hepatotoxicity: an analysis of 570 cases notified to the pharmacovigilance over a 13 years period

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Introduction: Liver-injury can be related to drugs. This diagnosis can be retained unless we eliminate infectious, obstructive, metabolic and immunologic diseases which are more common. We analysed notifications to the Tunisian national centre of pharmacovigilance, of suspected hepatotoxicity in order to identify the difficulties to attribute to the drugs the liver injury, the clinical features of drug-induced liver injury and the most involved drugs in liver injury.
Methods: All spontaneous reports of suspected drug-induced liver injury between December 1990 and December 2003 were analysed by applying the Council for International Organisations Medical Sciences scale and using the French method of assessment. We excluded from the study the cases with insufficient data, the cases with physiologic variation of hepatic tests, the cases with elevation of hepatic tests not confirmed with a second test and the cases of therapeutic advices. Clinical features were reported and included asthenia, jaundice, dark urine and abdominal pain.
Results: We identified four groups: group 1 (122 cases) in which the relation between the drug and the liver injury was certain, group 2 (175 cases) and group 3 (128 cases) in which the relation was doubtful with different degrees of indecision and group 4 (135 cases) in which the role of the drug was excluded. So the drug aetiology was retained or excluded in 46% of the cases.
The lack of data concerning biologic follow up and investigations performed to assess other aetiologies than drugs was more common in groups 2 and 3. The determination of these data could have permitted to improve the imputation score in 69% of the cases in groups 2 and 3.
Clinical features of drug-induced liver injury cases (group 1) were not different significantly from liver injury cases which were not induced by drugs (group 4).
Drug induced injuries were essentially acute: 73.8% of the cases in group 1. The remaining cases had an unknown evolution. The type of drug induced injury was mainly hepatocellular: 72.9% of cases in group 1. The other cases were cholestatic in 15.6%, mixed in 9% and unprecise in 2.5%.
Antibiotics were the drugs the most involved in the group 1 (68 cases over 122). The anti-tuberculosis drugs were the antibiotics the most incriminated (55 cases) and mainly isoniazid (40 cases).
Conclusion: In spite of the lack of data in the spontaneous reports, we could retain or exclude with certainty, in about half of the cases, the drug-induced liver injury aetiology.

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Propofol infusion syndrome, about one case

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Introduction: Propofol infusion syndrome (PRIS) is a rare and often fatal syndrome described in critically ill patients undergoing long-term propofol infusion at high doses. The main features of the syndrome consist of cardiac failure, rhabdomyolysis, severe metabolic acidosis and renal failure.
Methods: We describe the case of a young adult who developed a PRIS in October 2006.
Results: This case concerns a 17-year-old-female patient who was hospitalised for refractory generalised epilepsy. She received propofol in association with midazolam, which permitted the improvement of clonisms. Then, she presented at first hypotension requiring noradrenalin. In a second time, she developed hyperdiuresis, then renal failure and anuria, metabolic and lactic acidosis, rhabdomyolysis. Creatine kinase and myoglobin levels were increased. The propofol was stopped after 72 hours of treatment and replaced by thiopental. A PRIS was evocated. On the third day, bradycardia and anuria were noted and the hemodynamic situation worsened. An extracorporeal membrane oxygenation (ECMO) was performed urgently, in association with dialysis. The ECMO could be stopped after 6 days and a treatment with levetiracetam, oxcarbazepin and pregabaline was started.
In 2003, Vasile and al. described the pathophysiology of PRIS. The increase of creatine kinase and myoglobin could be interpreted as a proof of the direct necrotising effect of propofol on peripheral and cardiac muscles. On one hand, direct inhibitory effects of propofol diminished cardiac contractility. These mechanisms may be involved in the lack of response to catecholamine and in the need for escalating inotropic support observed. On the other hand, catecholamine could be responsible for decreasing the therapeutic effect of propofol. The negative inotropic effect of propofol, resulting in increased catecholamine requirements, could create a vicious circle, in which propofol and catecholamine drive each other in a progressive myocardial depressive effect.
Conclusion: In the present case, the outcome was favourable with a complete neurological recovery. Incidence of PRIS is less frequent in adult than in children and the evolution of most of cases described in literature was death. An early diagnosis is necessary as well as a precise knowledge of risk factors (prolonged infusion of high doses of propofol in patients with severe head injury, status epilepticus), in order to stop propofol infusion and to start adequate minimum fare.

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Sulfonamide cross-réactivity: a case report

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Introduction: The cross reactivity between Sulfonamides is discussed. We present a patient who develops hypersensitivity reaction to both: hypoglycemic sulfonylurea and diuretic sulfonamide.
Methods: This case was notified in regional center of pharmacovigilance of Sfax, (Tunisia) an inquiry of pharmacovigilance has been realized according to French imputation method.
Results: A 50- year-old woman had been treated by diltiazem since 2000 for hypertension, and gliquidone, since 2004, for diabetes.
In September 2006, she had taked allopurinol for gout. Twenty days later, she had developed hypersensitivity reaction (fever, generalized cutaneous eruption with pruritis and abdominal pain). All biologic explorations and abdominal echography had been normal. These symptoms were resolved 7 days after stopping all treatment.
The rechallenge of gliquidone leaded to the same symptoms. 10 days after curing, she had taked indapamide and she had developed a generalized pruritis after 1 hour.
The responsibility of gliquidone was strongly suspected in the genesis of hypersensibility reaction in this case. The score of imputability has been evaluated at I3 (C3S2) B3 (vraisemblable).
Conclusion: The pruritis developed after taking indapamide suggest a cross reactivity between sulfonamides. One must always keep in mind the possibility of cross-reactivity when using sulfonamides.

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Dry cough induced by haloperidol: a case report

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Introduction: Haloperidol is a neuroleptic drug. It is an powerful inhibitor of dopaminergic receptors. We describe a case of severe cough induced by haloperidol. At our knowledge, it was the first case.
Methods: This case was notified in regional center of pharmacovigilance of Sfax, (Tunisia). An inquiry of pharmacovigilance has been realized according to French imputation method.
Results: A 37-years-old man treated since 1998 by haloperidol, chlorpromazine and heptaminol for psychosis. Six years later, he was developed a non productive cough resistant to antitussive durgs. All explorations, particularly thoracic TDM, were normal. The cough was resolved 4 days after stopping haloperidol, despite the pursuit of the other drugs. The rechallenge of haloperidol reproduced the same symptom. So, haloperidol was definitively stopped.
The responsibility of haloperidol was strongly suspected in the genesis of the cough. The score of imputability had been evaluated at I3 (C3S2) B2 (vraisemblable).

This adverse effect had not been described with haloperidol. But the cough reflex seems to be modulated by many receptors: Dopaminergic, serotoninergic and sigma receptors.
Conclusion: When patients consult for cough, clinicians should think about drug induced aetiology, particularly when other aetiologies are eliminated.

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Fluoroquinolone psychiatric adverse effects: review of French pharmacovigilance data base
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Introduction: Psychiatric adverse effects of fluoroquinolones are known for a long time, but sometimes may be misdiagnosed with damaged patients outcome. We analysed the spontaneous reports to the French Pharmacovigilance system, with the aim to identify risk factors.
Methods: Reports of psychiatric adverse effects in which a fluoroquinolone was suspected, reported between June 2002 and June 2006, were extracted from the pharmacovigilance data base. Patients were classified in two groups, below and above 65-years old. For each report, gender, adverse effect, seriousness, time of onset and duration, fluoroquinolone involved, dose and evolution were recorded.
Results: A total of 258 cases have been reported concerning 142 women and 116 men. Mean age was 76.3 years. Five fluoroquinolones ciprofloxacine, ofloxacine, pefloxacine, ofloxacine and moxifloxacine have been involved. A significant difference was observed between the two groups concerning the frequency of adverse effects. In younger patients, the most frequently named adverse effect was epilepsy (22.41%), followed by mental confusion (20.69%) and hallucinations (19.83%). In older patients, mental confusion (51.72%) was first reported, than epilepsy (34.48%) and euphoria (25%) were noted. In both groups, moxifloxacine is the most involved fluoroquinolone, before ciprofloxacine. High or cumulated dose were noted. The average time of exposition to the drug before onset of the adverse effects was 5.21 days and symptoms lasted from 10 minutes to 28 days. Drug associations were found for 49.14% of the reported cases. The evolution was favourable for 92.64% of patients often after dechallenge. Seven deaths were observed, but none was attributable to the antibiotics. Differences between the chemical structures of the involved fluoroquinolones and pharmacokinetic specificities may explain the frequencies of psychiatric adverse effects and difference between the two groups.
Conclusion: The number of cases reported during this period remains moderate and methodological bias such as differences in fluoroquinolone indications, prescriptions and selling may limit our analysis. However, the pharmacological characteristics of fluoroquinolones, possibly associated with adverse effects may be promoted. The dose adjustment to renal function seems the first target for practitioners.

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Analyze of users' expectations of a hospital network system in risk management
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Introduction: The risk management is not only necessary but mandatory for all health care establishments. To improve safety and quality of care, regulatory authorities organized vigilances upon nosocomial diseases, drugs, medical devices, blood components and other medicinal or biological products and technologies of diagnosis. For a long time our hospital gathered in a 'comity of vigilances' the responsible persons for each regulatory vigilance. The aims of the comity are the improvement of the communication between vigilances and users and the promotion of the safety background. The hospital network system seems to be particularly adapted for these objectives. However a tool is efficient only when answers met to the users' expectations. So we performed a study to identify the needs of the medical and paramedical users.
Methods: A questionnaire was sent to the medical and paramedical users identified in the computered mailing lists of the hospital. The identification of profession, gender, age, job experience was required. The knowledges on each vigilance system, so as targets of the vigilance, regulations, facilities to identify the responsible persons and to contact them were analyzed. The whishes and expectations of the users were collected.
Results: A total of 520 mail-readers were identified and 237 (46%) answered to the questionnaire. Medical users and nurses represented 30% of each one, and technical users 30%. Others were pharmacists, dentists and midwifes. People were often between 30 and 50-years old, and worked for a long time in the hospital (>10 years ago). The comity is known by pharmacists, nurses and at last by practitioners. Hemovigilance, materiovigilance, pharmacovigilance and nosocomiovigilance are well identified. New vigilances or specific target so as reactovigilance have a restricted impact. The users knew guidelines and notification's liability but need more informations on contacts. and procedure Two third assessed the need of computered tools and expected an improvement of the application design and ergonomics. They asked opportunities for extensive connection for all hospital staff.
Conclusion: Despite methodological biases were identified, the questionnaire's replies and comments proved the needs of knowledge on risks and vigilances. It anticipated the expected involvement of the medical and paramedical staff. The ergonomics takes an important place in network system acceptability and efficacy.

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Antipneumococcal vaccine: inefficacy in a splenectomised patient
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Introduction: A 32-year-old female, who suffered from autoimmune hemolytic anemia, underwent splenectomy in 2001. She developed fatal pneumococcal sepsis with purpura fulminans 10 days after antipneumococcal booster with PPV23 in September 2006. French recommendations for revaccination indicate a delay of 5 years after primo-immunization. But vaccinal protection in splenectomised patients is very uncertain, and the protective antibody levels remain undefined. In front of numerous similar case reports in the literature, we would highlight the need to revise the procedure of protection against pneumococcal disseminated infections in postsplenectomy.
Methods: We reviewed international literature to find similar case reports and recommendations on immunization and prophylaxis measures after spleen ablation. Data on immunological response and antibodies levels after vaccination were also collected.
Results: A lot of overwhelming postsplenectomy infections was reported. *Streptococcus pneumoniae* was involved in more than 60%. A rapid progression to death occurred in at least 50%. Generally the primary immunization was administered 1–2 weeks before elective ablation or the day of resection in emergency cases. Concerning boosters, some authors recommended a single dose after 5 years, others a delay of 3–8 years, or still according to immune status. A few studies tried to establish the best frequency for checking antibody levels. Dosage methods were not homogeneous. Authors highlighted the fact that asplenic patients may lose their antibodies faster than healthy people, so that they should require booster earlier and not arbitrarily every 3, 5 or 8 years. By now, the French Pharmacovigilance Database had never registered fatal sepsis attributed to PPV23 inefficacy. Both assumptions could explain our case report: either the patient had no specific antibodies anymore and the delay of revaccination was inadequate; or the polysaccharides contained in the booster involved the consumption of the antibodies remaining from the primary vaccination. Anyway, PPV23 questions on its efficacy in immunocompromised patients, because it is T-cell independent and not thought to induce memory.
Conclusion: We conclude that up-dating the delay of antipneumococcal vaccination and boosters is a necessity for preventing overwhelming postsplenectomy infections due to pneumococcus. The frequency of antibody titration and the method of dosage have also to be clearly defined, so that the immune status of the patients could be easily follow-up.

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Hospitalizations for adverse effects related to the use of drugs in ambulatory: retrospective study of the cases declared in the regional centre of pharmacovigilance of marseille over the year 2005
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Introduction: In order to answer to one of the objectives of the regional plan of public health, the Regional Centre of Pharmacovigilance (CRPV) realized a retrospective study over the year 2005. The adverse effects related to ambulatory drugs and responsible of the hospitalization of patients were indexed and studied. The study was limited to three 'départements' (total 2 545 963 inhabitants).
Methods: This study was based on the information found in the registers and the files of the CRPV. For each studied case, various elements were taken into account, for instance the therapeutic class, the gravity of the adverse effect, the avoidable or not-avoidable nature (according the SCP but without taking account the treatment indication) and the iatrogeny.
Results: On 1657 adverse effects declared at the CRPV in 2005, 362 cases were eligible. The not-avoidable adverse effects are more frequent (76%) than avoidable adverse effects (24%). The most represented therapeutic classes are the anti-thrombotic drugs (21%), the anti-infectious drugs (20%) and the cardiovascular drugs (11%). The avoidable adverse effects are more often due to an error of the patient (38% of the cases). 33% seem to be related to the medical prescription and 5% on the pharmaceutical act. Lastly, 7% remain unspecified and 17% non-informative.
Despite all the studies and campaigns of information, the anti-thrombotic drugs remain at the first place of the avoidable serious adverse effects. Analgesics antipyretics and antispasmodics seem to be a target privileged for campaigns of prevention too, because the majority of the adverse effects due to products of this class are related to self medication. These results have to be balanced because they are based on the number of declared adverse effects and not on the number of occurred adverse effects. The non informative cases are far from being negligible (17%) and could skew the results. In addition, the role of the pharmaceutical act is probably undervalued.
Conclusion: Even if many adverse effects are considered as non-avoidable in this study, certain therapeutic classes seem to be privileged target regarding the action of public health, particularly the anti-thrombotic drugs and the class of analgesics, antispasmodics, antipyretics.

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Erythema multiforme induced by phenobarbital and radiotherapy
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Introduction: Erythema multiforme is a serious and life-threatening adverse drug reaction. Responsibility of drugs in the genesis of this entity is described in 20% of cases. Among them, barbiturates are most frequently responsibles. Radiotherapy may also cause erythema multiforme. We report a case of erythema multiforme induced by Phenobarbital and radiotherapy.
Methods: A 39-year-old woman had a breast carcinoma. She was treated by phenobarbital (10 cg daily) since 20th November 2001 for convulsive crisis secondary to cerebral metastases. On December 2001, she was treated with radiotherapy. A few days later, she was developed a generalized cutaneous eruption with pruritus and target skin lesions developed mainly on the palmo-plantar region. All symptoms and signs are resolved 3 weeks after stopping phenobarbital and radiotherapy.
Results: An inquiry of pharmacovigilance has been realized according to French imputation method. It has allowed to suspect strongly the responsibility of phenobarbital in the genesis of erythema multiforme in this case. The score of imputability has been evaluated at I2 (C2S2) B3 (plausible). Erythema multiforme associated with barbiturate's treatment and radiotherapy is described in literature. When barbiturates and radiotherapy are given simultaneously, they may act synergistically to increase the risk of development of this adverse effect. The pathogenesis of this association is not well known. It seems to be an immunology reaction.

Conclusion: Practitioners prescribing phenobarbital should be informed of this risk and should inform pharmacovigilance center if such symptoms appear.

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Cohort study of serious adverse side effects after influenza vaccination in old people's home in the departments of Loire Atlantique and Vendée

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Introduction: Influenza vaccination (IV) is usually recommended in autumn and winter for the prevention of flu for elderly people (>65-year old), people exposed at work and those who have chronic illness such as diabetes. The most common adverse side effects are local reactions (oedema, erythema, pain and induration) or general reactions, like fever, discomfort, shivers, muscle and articulation pain. These adverse side effects are frequents but not serious. This year, four cases of deaths occurred in Israel after the IV have stimulated a special monitoring of serious adverse side effect after IV by the French Drug Agency (Afssaps). However no causal relationship between these deaths and the flu vaccination was proved.
Methods: Nurses, qualified professionals or physicians of 377 old people's home in the departments (approximate population of 22 620 people) of Loire Atlantique and Vendée have been contacted by telephone between the 13/11/06 and the 1/12/06 to collect serious adverse side effects which have occurred in the eight days after vaccination and the eventual glycaemic perturbation in the diabetic population. We have phoned people and have send a courier resuming the purpose of this collect and a declaration formulary specially edited for 2006 flu campaign. Consequently, we hope that the rate of considering reply would be high.
Results: On the approximately 22 620 residents, more of 90% have got vaccinated, the others have refused vaccination or used homeopathy vaccination. Only eight declarations have returned to regional pharmacovigilance centre of Nantes. Among these reports, only four have concerned a serious adverse side effect (death, acute pulmonary oedema, stroke and death, angioedema). No glycaemic trouble had been reported.
Conclusion: Influenza remains an important cause of illness and death in our country. Influenza vaccination has been associated with reduced risk of hospitalisation for cardiac and cerebrovascular disease, reduced risk of recurrent myocardial infarction and fewer hospitalisations for individuals with diabetes. In our study, only few cases of serious adverse side effects have been collected. Findings from our study suggest that influenza vaccine is not only effective but also safe in the elderly population. So the health professionals can easily convey the message to their elderly patients that immunizations are an important part of their care.

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Increased serum testosterone related to phenylbutazone therapy: a drug-hormone interaction

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Introduction: Elevated serum testosterone may be caused by exogenous androgens, androgen-secreting tumours, mutations of androgen receptors or pituitary adenomas. Only one report described spurious elevations of testosterone (DPC RIA, Los Angeles, CA, USA) among five patients (four males) with ankylosing spondylarthritis taking phenylbutazone. We report three new observations of this seemingly rare drug-hormone interaction.
Methods: We used other testosterone assays (DSL RIA, Webster, USA and BRAHMS TRACE on KRYPTOR automate, Berlin, Germany) than in the previous publication. We also determined serum testosterone by tandem mass spectrometry. Steady state serum levels of phenylbutazone and its main metabolite oxyphenbutazone were measured in all three cases.
Results: A 46-year-old man received phenylbutazone (300–500 mg daily) and sulfasalazine for more than 10 years for a HLA B27 positive ankylosing spondylarthritis. His gonadal function was clinically normal. He denied any intake of exogenous androgens. Serum testosterone was first determined in October 2000, amounting 154 nmol/l (DSL RIA; N: 9.4–37 nmol/l). FSH and LH, TSH, cortisol and prolactin levels were normal. cerebral and abdominal TDM examinations were normal. This extremely high testosterone was confirmed twice in 2001 with the same assay, and on September 2005 using TRACE method on KRYPTOR (53 nmol/l; N: 6.5–24 nmol/l). On January 2006, phenylbutazone was stopped; 24 days thereafter, serum testosterone returned to normal (15.8 nmol/l by TRACE). As his disease was poorly controlled, phenylbutazone was resumed; 15 days later, testosterone reached 46 nmol/l (positive rechallenge). Two other patients without hyperandrogenism had high serum testosterone while on phenylbutazone: a 30 year old man with a serum testosterone of 38.7 nmol/l (TRACE) 7 days after initiation of the drug, and a 35-year-old woman with a testosterone level of 21.5 nmol/l (TRACE; N: 1.5–2.94 nmol/l). In all three cases, serum phenylbutazone and oxyphenbutazone were within normal range. *In vitro*, addition of an 8-fold range of phenylbutazone concentrations including therapeutic levels to the serum of three healthy volunteers increased testosterone by 250%; addition of oxyphenbutazone had no effect.
Conclusion: Many patients with ankylosing spondylarthritis are still using phenylbutazone. Therefore, physicians should be aware of this drug-hormone interaction. The mechanism might be a common epitope shared by phenylbutazone, a non-steroidal drug (but not oxyphenbutazone) and testosterone with antibodies used to perform several testosterone assays, or elsewhere an interference of phenylbutazone with the tracer. This drug-hormone interaction might be frequent if searched for systematically.

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Hypoglycaemia associated with the use of angiotensin II receptor antagonists: a signal generated by sole confounding

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Introduction: Automated disproportionality analysis of spontaneous reporting is increasingly used routinely in pharmacovigilance systems. However, most of these methods are unable to fully investigate potential biases such as confounding one. The potential role of confounding in safety signal generation has already been demonstrated in situations where the prior publication of a possible association was also involved [angiotensin converting enzyme (ACE) inhibitors and hypoglycaemia]. We wanted to study the potential effect of confounding on automated safety signals generation in a situation where no notoriety effect would be expected.
Methods: The French Pharmacovigilance database was analysed for an association between adverse drug reaction reports mentioning hypoglycaemia and angiotensin II receptor antagonists using the case non-case method. The association between angiotensin II receptor antagonists or other chosen drugs and hypoglycaemia was also tested in the subgroups of patients taking or not antidiabetic agents.
Results: Hypoglycaemia was found in 807 of all 174 595 reports in the database and in 589 of 6909 reports with antidiabetic agents (OR 69.2; 95% CI 59.2–81.1). It was found in 33 of the 4.155 reports involving angiotensin II receptor antagonists (OR 1.8; 1.2–2.5). Other study drugs associated with hypoglycaemia were ACE inhibitors (OR 3.0; 2.2–4.2), disopyramide (OR 17.4; 10.4–29.1), cibenzoline (OR 107.3; 77.8–148.1), atenolol (OR 2.0; 1.2–3.6), dihydropyridines (OR 1.9; 1.2–3.0), frusemide (OR 2.8; 2.3–3.5), but not diazepam, verapamil, combination thiazide diuretics. However, angiotensin II receptor antagonists and other drugs were associated with antidiabetic agents. The association of angiotensin II receptor antagonists with hypoglycaemia disappeared in the subgroups of patients taking or not antidiabetic agents, (OR 0.4; 0.2–0.8 and OR 1.4; 0.8–2.7) respectively). Association of hypoglycaemia with disopyramide or cibenzoline was not affected.
Conclusion: Although there is no evidence in the literature supporting an association between hypoglycaemia and the use of angiotensin II receptor antagonists, a signal was generated in the French pharmacovigilance database. This signal for which no notoriety bias could be suspected disappeared after stratification on antidiabetic agents use, thus demonstrating the role of confounding by indication in its generation.

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Necrosis of the intranasal structures and soft palate as a result of nasal inhalation of heroin in two patients

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Introduction: The link between intranasal cocaine abuse and necrosis of nasal, paranasal sinus and palate structures is well documented. In contrast, few data are available concerning nasal mucosa necrosis following opioid inhalation. We report here orofacial lesions in two patients with chronic heroin inhalation.
Methods: These two cases were notified to the evaluation and information on pharmaco-dependence centre of Montpellier. The link between orofacial lesions observed and the intake of heroin has been made according to the data of the literature.
Results:
Case 1. It concerns a 36-year-old man with a history of 6 years heroin nasal inhalation only. In November 2006, he consulted to the emergency unit because of dysphagia and suppurating sputum. The exam showed complete nasal septum necrosis with saddle nose deformity and soft palate perforation. Samples performed showed Candida parapsilosis. The patient was treated with antibiotics and antifungals (fluconazole), waiting for surgical reconstruction. Methadone maintenance therapy was started. HIV, HCV and HBV serologies were negative. Heroin was diluted with acetaminophen and caffeine. Urinary screening was positive for opioids and negative for cocaine.
Case 2. It concerns a 24-year-old woman. She started to take heroin by nasal and intravenous route in 2002. In August 2006, she presented with phlebitis on her upper limb; so intravenous heroin was stopped. In October 2006, she complained from nasal pain. At the exam, nasal septum and sinusonasal wall necrosis with suppurating rhinorrhea was found. HIV, HCV and HBV serologies were negative. Urinary screening was positive for opioids and negative for cocaine. Antibiotics were prescribed with nasal wash out and methadone maintenance therapy was started with a good result.
Conclusion: Even if intranasal necrosis has been rarely reported with heroin inhalation, several cases have been described in the literature with other opioids: hydrocodone, crushed oxyContin. The mechanism of tissue necrosis with opioid abuse remains unknown. One possible explanation may lie in the effects of opioids on the immune system. Opioid drugs may exert immunosuppressive effects through the inhibition of cell-mediated immunity, allowing for the development of invasive bacterial or fungal infections.

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Prospective clinical and biological follow-up of three breastfed babies from azathioprine-treated mothers

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Introduction: Azathioprine is an immunosuppressive drug used for preventing organ transplant rejection and treating various chronic and autoimmune diseases. Breast feeding during maternal azathioprine treatment is usually contraindicated mainly because of the lack of data on its transfer into breast milk resulting in the ill-evaluated potential risk for the baby of developing adverse effects. We report the outcome of three breastfed babies from mothers treated with azathioprine.
Methods: Three azathioprine-treated mothers were recruited when they or their doctor questioned Lyon Pharmacovigilance Center prior to breast feeding. The clinical follow-up of the babies was performed via telephone interviews of the mother and/or the pediatrician. The three babies had a blood cell count at birth. Further biological tests in two babies consisted of blood cell counting, 6-thioguanine nucleotide (6-TGN) and 6-methylmercaptopurine nucleotide (6-MMPN) assay at birth and/or at 3 weeks of age, and thiopurine methyltransferase (TPMT) phenotyping.
Results: Two mothers were treated for inflammatory bowel disease and one for systemic lupus erythematosus with 100, 150 and 175 mg of azathioprine, respectively combined with prednisolone in the first case, and infliximab in the second case. The first baby was breastfed during 12 months and the other two babies for up to 4 months. The babies' blood cell counts remained within normal

range either at birth or at 3 weeks of age. The baby's blood cell count at 3 days of life found a low concentration of 6-TGN and undetectable 6-MMPN. In addition, neither 6-TGN nor 6-MMPN could be detected at 3 weeks of life in the same baby and in another one. TPMT phenotyping in two babies showed normal enzyme activity. The clinical follow-up was maintained regularly until the babies were 24-, 22- and 4-month old, respectively. They all were healthy with normal growth rate and no history of recurring infections at the termination of follow-up.
Conclusion: These three cases suggest that breast feeding during maternal azathioprine therapy may be safe. These results may probably be extrapolated to mercaptopurine, the active metabolite of azathioprine. However, further studies are needed to assess the short- and long-term safety of both azathioprine and mercaptopurine during lactation.

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Colchicine-induced pancytopenia during therapeutic dose administration. French pharmacovigilance database survey and literature review
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Introduction: Colchicine is an antimitotic agent, highly effective in the treatment of microcrystalline arthritis, Behcet's disease and familial Mediterranean fever. Pancytopenia by bone-marrow depression is common after colchicine overdose and intoxication. It is less common at therapeutic dose but it may be fatal. Colchicine has a low narrow therapeutic range. The purpose of this work is to determine the seriousness, the risk factors and the outcome of pancytopenia in patients treated with therapeutic dosage of colchicine (≤2 mg/d).
Methods: All case-reports of pancytopenia and marrow depression collected in French Pharmacovigilance database between 1984 and May 2006, and from literature, were analysed.
Results: Forty-two case-reports were retained: 33 from database and nine from literature.
Colchicine is primarily eliminated through biliary excretion. Renal excretion plays a less significant role. Case reports suggest that pancytopenia often occurred during colchicine administration with combined liver and/or renal impairment (database: 81.8%, literature: 44.4%), and with hematotoxic drugs (database: 54.5%). Co-administration of colchicine and CYP3A4 and/or P-gp inhibitors may impair colchicine elimination, resulting in excess drug exposure and toxicity, explaining severe adverse effects and deaths.

	Database $n = 33$	Literature $n = 9$
Average age	63.8 (33–93 years old)	61.9(29–96 years old)
Renal/liver impairment	$n = 27$	$n = 4$
Drug-drug Interaction	$n = 3$	$n = 2$
P-gp inhibitors	$n = 6$	–
Hematotoxic drugs Comb	$n = 18$	–
Onset delay ≤1 month	$n = 19$	$n = 7$
>1 month	$n = 9$	$n = 2$
unknown	$n = 5$	–
Outcome Favourable	$n = 13$	$n = 4$
Sequelae	$n = 2$	–
Death	$n = 14$	$n = 5$
Unknown	$n = 4$	–

Conclusion: Colchicine can be toxic even at therapeutic dose. It should be used with extreme caution in patients receiving CYP3A4 and/or P-gp inhibitors and hematotoxic drugs, particularly if they are elderly with renal and/or liver impairment. We propose modifications of the French summary of product characteristics concerning adverse drug reactions section.

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Pharmacovigilance in clinical trials: a tremendous change
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Introduction: The French law of 9th August 2004 comes into force on 27th August 2006. It implements the Clinical Trials Directive (2001/20/EC) into French regulation. This Directive aims at harmonising the regulatory environment for clinical trials on medicines in Europe. Its effect is to adopt internationally recognised principles of good practice. This new regulation strengthens both investigator and sponsor's responsibilities. The investigator must report immediately any serious adverse event and assess the causality of trial medicine. The sponsor now becomes clearly responsible for the ongoing safety evaluation of the investigational medicinal product.
Methods: To comply with the new pharmacovigilance rules, the sponsor needs to make arrangements to record, notify, assess, report, analyse and manage adverse events in those trials. The regulations distinguish between adverse events, serious adverse events, serious adverse reactions defined as reasonably related to trial medicine and Suspected unexpected serious adverse reactions (SUSARs). The trial sponsor must assess the causality also and the expectedness (is the reaction a recognised adverse effect of the medication or is it unexpected?) of adverse events.
Results: Sponsors must report electronically SUSARs to both EMEA through the European pharmacovigilance database EudraVigilance, the AFSSAPS and the relevant Ethics Committee. The regulations set time limits. Fatal or life threatening SUSARS: not later than 7 days after the sponsor had information that the case fulfilled the criteria for a fatal or life threatening SUSAR, and any follow-up information within a further 8 days. All other SUSARs: not later than 15 days after the sponsor had minimal information required. The sponsor must also send an annual safety report on all serious adverse reactions (including SUSARs) to the AFSSAPS and relevant Ethics Committee. Thus, this report provides an overview of subject's safety and a benefit-risk evaluation on a regular basis. Any other safety issues that might alter the current benefit-risk assessment of the trial medicine are submitted to expedited reporting by the sponsor.
Conclusion: New regulations will better protect the rights, safety and well-being of patients taking part in clinical trials of medicines. Moreover, EudraVigilance contributes to the protection and promotion of public health in Europe and is a powerful tool to monitor the safety of medicinal products and in minimising potential risks related to suspected adverse reactions.

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Choice of the comparator for signal generation in pharmacovigilance databases: impact on detection thresholds
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Introduction: Many statistical methods are used for automated detection of signals in pharmacovigilance, mostly based on disproportionality measures. They compare the relative reporting of one event for one drug to reporting for all other drugs. This comparator seems appropriate when it does represent the baseline risk of reporting. This might not be the case when it includes drugs known to be involved in the occurrence of a specific event. We wanted to study, for several events the effect of removing reports concerning drugs involved in particular events on the detection thresholds of signals for other drugs, newly marketed or older.
Methods: We used the case non-case approach to estimate detection thresholds for new or older drugs in the French Pharmacovigilance database considering different frequency of reporting. We studied cases reports between October 2005 and September 2006. Events considered were bleeding, headache, hepatitis, toxic epidermal necrolysis (TEN), myalgia, myocardial infarction and stroke.
Results: During the study period, 19 173 reports were registered in the database, among which were: 908 bleeding cases, 361 headache cases, 548 hepatitis cases, 49 TEN, 369 myalgia cases, 53 myocardial infarction cases and 321 stroke cases. For bleeding, the number of reports of interest (detection threshold) for new drugs with 50 reports all events considered, was estimated at six when compared to all other drugs and at three after excluding reports concerning the main drugs already associated with bleeding. These thresholds were decreased for all reporting frequencies studied for bleeding and stroke. They were not modified for headache, hepatitis, TEN, myalgia or myocardial infarction. Thresholds were similar whether they were evaluated for a new drug or for an existing one.
Conclusion: The choice of the comparator is of great importance for signal generation and has great influence on detection thresholds. The inability to define a specific comparator might limit the performance of data mining when it is used to generate signals without prior hypotheses. For orientated signal exploration, the comparator must be defined, especially for serious, specific and frequent adverse events.

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Adverse drug reactions in children: notifications analysis reported in a French pharmacovigilance centre
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Introduction: According that drugs are poor evaluated in children with many prescriptions off license, pharmacovigilance is the only way for a safer use of these drugs. The aim of the study was to assess spontaneous notifications in pediatrics patients.
Methods: Case reports concerning adverse drug reactions in children from 0 to 18 years old in a French pharmacovigilance centre over a 6-year period (2000–2005) were analyzed. All the notifications were taken from the French pharmacovigilance database. The suspected drugs were classified according to the Anatomical-Therapeutical-Chemical classification. The adverse drug reactions were classified according to the WHO nomenclature. Informations about the origin of notifications were collected from local database.
Results: During this period, 215 adverse drug reactions for 126 children were reported. Most of case were reported from hospital health professional (65%), with 45% from University hospital. The absolute number of reports was higher in children under 2 years of age (35%). There were 54% of serious effects and 42% of non serious. The most commonly affected organ-systems were general disorders, central nervous system and the skin. The therapeutic groups most commonly involved were anti-infective agents and vaccines (40%), central nervous system drugs (28%), metabolism and digestive tract drugs (8%). Among the overall cases reports, 11% were linked to a medication error or a misuse (nine accidental overdoses, one drug misuse, one diagnostic error, three voluntary drug intoxications). We evaluated incidence of adverse drug reactions in children hospitalized in university hospital about 1.3%.
The results concerning age of children, affected organ-systems and therapeutic groups involved agree with many published studies. In the literature, the overall incidence of adverse drug reactions in hospitalized children is about 5–10%. Then, we estimate reporting rates between 1.3% and 2.5% for all adverse drug reactions, and 10% for serious events.
Conclusion: Spontaneous notifications concern frequently very young children, this reflects a higher susceptibility and a lack of pharmacological studies. Increased reporting of adverse events might improve a safer use of drugs in children.

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Hyponatremia as an adverse effect of recommendations during heat wave?
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Introduction: After the exceptional heat wave occurred in France in 2003, recommendations were edited in order to prevent hospitalisations and reduce morbi-mortality. One of them was to increase hydric intakes, particularly for young and old people. However, hyponatremia is described as a possible effect of excessive hydratation, especially for old people taking drugs known to induce such an adverse

drug reaction (ADR). Several cases of hyponatremia in old people taking thiazidic drugs and incited to drink a lot have been reported in the literature. We wanted to evaluate potential adverse effects of recommendations taken in 2003. The aim of this work was to compare cases of serious hyponatremia reported in the French Pharmacovigilance Database during summer 2003 and summer 2006.
Methods: All serious hyponatremia cases reported to the French Network of Pharmacovigilance between 1 July and 31 August 2003 and between 1 July and 31 August 2006 (two periods with heat wave) were analysed with respect to age, gender, drugs involved, evolution as well as drug imputability. Comparisons between cases were made using chi-2 square tests for qualitative variables, and *t*-tests for quantitative variables.
Results: The total number of serious hyponatremia cases registered into the French Pharmacovigilance Database was similar in summer 2003 ($n = 42$) and 2006 ($n = 44$). Mean age (75.4 vs. 78.5) and sex ratio (0.4 vs. 0.3) were comparable. During 2003, 73 drugs [58 possible (I1), 12 plausible (I2) and three likely (I3)] were involved. During 2006, more plausible cases were notified (37 'I1', 24 'I2', and 2 'I3'; $P = 0.02$). Main pharmacological classes involved were diuretics alone or in association (30.1% in 2003, 41.3% in 2006, NS), serotonin reuptake inhibitors (17.8% and 14.3%, NS), antiepileptics (6.8% vs. 7.9%, NS), and proton pump inhibitors (5.4% and 3.2%, NS). Clinical outcomes were similar in 2003 and 2006 (mainly favourable).
Conclusion: The main clinical characteristics of reports with serious hyponatremia were similar in 2003 and 2006. More plausible cases were notified in 2006, may be because such ADRs were expected in 2006, after the first heat wave in 2003. Despite recommendations taken after 2003, no excessive cases were notified to the French Network of Pharmacovigilance during a second heat wave in 2006.

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Inherited long QT syndrome revealed by drug to drug interaction
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Introduction: Although generally well tolerated, voriconazole, a recent marketed azole antifungal, may be associated with adverse events like transient visual disturbances, hepatotoxicity, and skin rashes. Cardiac adverse events, even though mentioned in summary of product characteristics are less frequently reported in the literature.
Methods: We report here a rare life threatening cardiac complication associated with voriconazole treatment, notified to our pharmacovigilance center.
Results: A 14-year-old girl with acute myeloid leukemia and a suspected mucormucosis infection was treated with intravenous voriconazole and caspofungin. Because of fungal infection worsening, voriconazole was switched to posaconazole. Eight hours after stopping voriconazole and 4 hours after a single oral dose of posaconazole, she presented with a QT interval prolongation with ?torsades de pointes' following by a 3 minutes cardiac arrest. Serum magnesium was 0.44 mmol/l, (N: 0.75–1 mmol/l). After recovery, the fungal infection improved, with caspofungin and liposomal amphotericin B.
According to the Begaud method, the causality link between drugs and ?torsades de pointes' observed was possible for voriconazole and dubious for all other drugs. Voriconazole plasma level measured 15 hours after the last administration of voriconazole was 7 mg/L (normal trough levels: 0.6 to 1.5 mg/L). Analysis of patient metabolism revealed that she was extensive metabolizer for CYP2C9 and CYP2C19. High voriconazole plasma level could be explained by interaction with omeprazole and/or posaconazole, two inhibitor of CYP450. Cardiac exploration showed inherited long QT syndrome, never observed before and probably revealed by voriconazole.
Conclusion: This adverse effect seems to be plurifactorial as this patient presented with several risk factors for cardiac complications: female gender, intravenous voriconazole with excessive plasma levels, drug–drug interaction, hypomagnesemia, and long QT syndrome. Serial electrocardiographic and electrolytes monitoring may be considered when voriconazole is administered in patients, in association with voriconazole therapeutic monitoring.

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Intracranial haemorrhage with favourable outcome in a highly treatment experienced female patient
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Introduction: The worldwide incidence of intracranial haemorrhage (ICH) ranges from 10 to 20 cases per 100 000 people, increases with age and is associated with high proportion of fatal outcomes[1, 2]. The incidence of ICH in the AIDS population in the pre- highly active antiretroviral therapy (HAART) is estimated to be 0.2%, a risk 25 times higher than in the general population[3].
Methods: We report a case of an HIV-infected female patient who experienced an ICH while participating in an open-label safety study to evaluate the safety of tipranavir/ritonavir (TPV/r) as part of a HAART regimen for the treatment of patients with HIV infection who had failed and/or were intolerant to combination antiretroviral (ARV) therapy and had limited treatment options.
Results: The patient was a 56-year-old HIV positive female patient who had a medical history of ischemic cardiomyopathy, acute renal failure due to venous thrombosis, oral candidiasis, peripheral neuropathy and *M. avium* infection, prior to starting TPV/r 500/200 mg bid on 01-Mar-04. The patient also received T-20 SC 90 mg bid. On 09-Jan-04, her viral load (VL) was at 125 000 copies/mL (cp/mL) and her CD4 count at 34 cells/mm^3. On 07-Apr-04, her VL was at 1079 cp/mL and her CD4 count at 135 cells/mm^3. On 05-Apr-04 the patient was hospitalized for severe fatigue. On admission, her platelet count (239 000 cells/mm^3) and PT (100%) were normal but her PTT (51/33 s, grade 1) was elevated while receiving calcium heparin therapy. On 08-Apr-04, she became comatose. CT scan revealed severe right temporoparietal haemorrhage. She had no history of alcoholism, recent trauma, or bleeding. Her ARV regimen was interrupted. On 13-Apr-04, the patient improved but had right hemiplegia. The ARV medications were reintroduced on 03-May-04. On 27-Sep-04, CT-scan showed regression of haematoma and mass effect. Hemiplegia progressively resolved. On 09-Nov-04, her CD4 count was at 212 cells/mm^3 and her VL was now <200 cp/mL. The patient remained on TPV/r and T-20, as of Nov-06, with a CD4 count at 535 cells/mm^3 and a VL at 86 cp/mL.
Conclusion: The incidence of ICH in HIV-infected patients treated with HAART needs to be investigated since safety information about ICH in patients receiving TPV/r was recently released. Risk factors may have become modified with the aging of the HIV-infected population and the use of medications which predispose a patient to bleeding. This patient's primary risk factor for ICH appears to have been heparin therapy, but a contribution by her HAART cannot be ruled out. Overall, HAART has a favourable benefit-risk assessment. Her physicians did not believe that tipranavir used was related to the ICH event and her treatment was associated with significant immuno-virologic efficacy.

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Effect of well-established drug-event associations on the generation of new signals in spontaneous reporting databases
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Introduction: Automated disproportionality analysis of spontaneous reporting is increasingly used routinely, but it may be influenced by the presence in the database of well-established drug-event associations which could hamper the generation of new signals. We wanted to explore the influence of well-established drug–event associations on the generation of new safety signals in spontaneous reporting databases.
Methods: Within 16 years of spontaneous reporting in the French Pharmacovigilance database (January 1986 to December 2001), disproportionality of reporting was tested before and after removing reports concerning well-established drug-event associations for six events of interest (gastro-intestinal haemorrhage, headache, hepatitis, myalgia, myocardial infarction and haemorragic stroke) using the case non-case approach.
Results: In the whole database, we initially identified 51 signals for gastro-intestinal haemorrhage. After removing reports involving NSAIDs, thrombolytic, anticoagulant and antiplatelet agents, six new signals appeared (incriminating isotretinoïne, thiocolchicoside, pentosane, rivastigmine and orlistat) and nine disappeared, concerning drugs frequently associated to the above-mentioned drugs in the removed reports. The same approach was applied to other events: after removing well-known drugs associated with each event of interest, for headache six signals appeared whereas three disappeared, for hepatitis 2 appeared whereas three disappeared, for myocardial infarction 1 appeared whereas none disappeared and for haemorragic stroke 2 appeared whereas 26 disappeared.
Conclusion: This study demonstrates that using the whole database as a comparison group for disproportionality analysis does not provide a good estimation of the baseline risk of an event when it includes drugs known to be associated with the event. Excluding these drugs from the comparison group could greatly improve the efficiency of automated methods of signal generation.

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Can spironolactone be a cause of neutropenia?
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Introduction: Despite wide use for many years, neutropenia is not listed in the adverse effects section of spironolactone monograph. Recent convincing reports to Lyon pharmacovigilance centre prompted us to review other available cases notified to the French pharmacovigilance network.
Methods: Cases of spironolactone-associated neutropenia were identified from the French pharmacovigilance database up to December 2005. Inclusion criteria were neutrophil count <1.5 g/L with otherwise normal hemoglobin level and platelet count, and spironolactone exposure at least during the 10 days preceding the biological diagnosis. Cases with other possible medical causes or clearly attributable to concomitant drugs were excluded for further analysis.
Results: Of 81 retrieved cases, 32 corresponded to these criteria. The population mostly consisted of female patients (sex ratio: 0.39) and the mean age was 68.8 ± 6 years. Neutropenia was identified on a routine blood cell count in seven patients (22%), or on the basis of clinical symptoms with moderate constitutional symptoms in nine patients (28%), fever with localized infectious complications in 11 (35%) and septicemia in two (6%) (circumstances not reported in three patients). Only four patients remained asymptomatic during the whole course of neutropenia. The time to neutropenia onset after spironolactone was started ranged from 0.5 to 24 months (mean: 213 days), and was lower than 3 months in 63% of patients. The mean number of circulating neutrophils was 0.34 g/L and 72% of patients had neutrophil counts less than 0.5 g/L. Results of bone marrow aspiration available in 19 patients evidenced neutrophil maturation arrest in 12, selective depletion of the myeloid series in 3, and was normal in 4. Although 44% of patients had at least two pejorative prognosis factors at diagnosis, they fully recovered within 40 days (complete outcome unknown in two cases). Spironolactone (mean dose: 56.7 mg) was used as a single ingredient in 19 patients and in fixed combinations with furosemide or altizide in 13. Spironolactone was the single suspected drug in only six patients. In the other 26 patients, a concomitant treatment was equally suspected on the basis of chronological data, and it was a recognized possible cause of neutropenia in 18.
Conclusion: Although only 14 of our cases fulfilled plausible criteria for spironolactone-induced neutropenia, we suggest adding spironolactone as a possible cause of neutropenia. In addition, our data are in accordance with the results of a recent Spanish case control study that identified spironolactone among the drugs most strongly associated with the risk of agranulocytosis [OR, 19.97 (95% CI, 2.3-176)] (Ibanez L et al, 2005).

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How to identify rare adverse drug reactions for pharmacogenic studies: example of Torsade de Pointes in France

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Introduction: Drug-induced *Torsade de Pointes* (TdP) is one of the most serious adverse drug reactions (ADRs) and has led to at least seven post-marketing drug withdrawals in the last decade. Most commonly, TdP is generally associated with a prolonged QT interval. Frequently, in the critical care setting, this prolonged QT interval is directly attributable to medications the patient is taking. These ADRs is very difficult to study because TdP which could lead to death of patients. The EUDRAGENE project is an European collaborative project which aims to establish a freely-shared case–control collection of DNA samples as a resource for studying genetic predictors of ADRs. We present the results of cases collection of TdP for EUDRAGENE, using the French Pharmacovigilance system and hospital medical databases.

Methods: Definition of cases for EUDRAGENE must reach the following inclusions criteria: a polymorphic ventricular tachycardia (VT) or a ventricular fibrillation (VF) documented by ECG in the case record, a QT interval greater than 440 ms in male and 450 ms in female and a current exposure to a drug previously associated with QT syndrome and exclusion of congenital LQTS, recent myocardial infarction, or known electrolyte disturbance. We searched in the French Pharmacovigilance Database cases between 2000 and 2005 cases with terms « Torsade de pointes » and « prolongation of QT». Relevant observation copies were asked to every pharmacovigilance centre. A second data source included medical records from five hospitals in the Southwest area of France between 2000 and 2004 using ICD-10th codes of VT and VF. The list was checked with medical records and discharge letter in order to select relevant cases.

Results: In the French Pharmacovigilance Database, 97 files were examined, 64 cases were excluded and 33 cases were possible (34%). In second data source (medical records), 793 files were examined. 758 cases were excluded and 35 cases were possible (5%). Finally, 22 patients agreed to take part in the EUDRAGENE project from medical records data source and five patients from Pharmacovigilance database.

Conclusion: The two systems are very effective to identify patients with very rare ADRs for pharmacogenic studies but hospital medical databases allow acsertainment of more cases than the French system of Pharmacovigilance because the most serious ADRs are notified and more patients are dead in Pharmacovigilance database. Consequently, we will develop a network of cardiologists to identify more rare ADRs for the EUDRAGENE project.

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Safety of avocado-soybean unsaponifiables: data from the French Pharmacovigilance database

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Introduction: Patients suffering from osteoarthritis (OA) are often tempted to try long acting drugs like chondroitin sulfate, diacerhein or avocado-soybean unsaponifiables. Although avocado-soybean unsaponifiables (ASU) are largely prescribed in France, there are few data concerning their post-marketing safety. Therefore, we analysed data provided from French spontaneous reporting system via the network of Pharmacovigilance centres.

Methods: We analysed all suspected adverse reactions concerning ASU reported between 1980 and 2004 and notified in the French Pharmacovigilance database.

Results: We kept 110 adverse drug reactions (ADRs) concerning 110 patients (mean age 68 years, 71% female). Half of ADRs were not serious (51%) and 34% resulted in hospitalisation or prolonged it. No death was reported. In majority of cases, causality of ASU was 'possible' and others drugs were also suspected. The most frequently reported ADRs were cutaneous disorders (33% of all ADRs) with eczemas or urticaria. Secondly, liver disorders (15.1%) were notified (mostly hepatocellular injuries). Thirdly, gastrointestinal disorders (n = 14; 13.2%) were notified, with nine cases of colitis and/or diarrhea. In these cases, dechallenge of ASU allowed to a rapid regression of symptoms. Others significant ADRs were: coagulation and platelets disorders (7.5%), neurological disorders (6.6%) and metabolism or nutritional troubles (4.7%).

Conclusion: Analysis of ADRs of ASU notified since their commercialisation highlighted the diversity of ADRs with a large type of class-organ concerned. Cutaneous, hepatic and gastro-intestinal disorders were the most frequently reported ADRs. As ASU is largely prescribed in France, incidence of their adverse reactions seems to be 'very rare' (although we did not take into account the part of under-notification). These safety data should be discussed with the poor expected clinical benefit of ASU in rheumatology (low 'Service Medical Rendu') or in stomatology (insufficient 'Service Medical Rendu').

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www.e-lactancia.org: internet source on breastfeeding, drugs usage and abuse substances

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Introduction: Paramount importance of breastfeeding is 90% of women who breastfeed get medication. Misinformation on the compatibility of drugs, phytotherapy, toxins, diseases and breastfeeding can lead to withdraw suckling unnecessarily. A web source with information on compatibility of medication, other substances and breastfeeding.

Methods: Several sources were consulted on medication transfer to breast milk, dyes, herbs, abuse substances, contaminants and preservatives. A database in Windows Access was created and a web application was built with. A searching device permits to retrieve data easily and improves its presentation.

Results: At www.e-lactancia.org we can enter a page displaying two searching windows: (i) active pharmaceutical principle (ii) type of substance. By following direction we can reach to a page with detail information on possible effects to the child, pharmacokinetics, colored alerts (green: safe, yellow: possibly unsafe, red: contraindicated) and alternative choice.
We have 1470 products listed at four lactation risk categories:

Category/colour	Risk, problem	Breastfeeding compatibility	N	%
0/Green	No	Yes	649	43.7
1/Yellow	Mild	Yes	506	34.0
2/Orange	Moderate	Assess*	248	16.7
3/Red	Severe	No **	83	5.6

*:Assess risk/benefit. Consider temporary interruption
**:Contraindicated, stop medication or breastfeeding

Conclusion: The project, which is the result of multidisciplinary work done by Pediatricians, Pharmacists and computing staff needs to be up-dated periodically. By the time, over 1470 different substances can be fund. We believe it is an efficient support to health workers on medication and abuse substances during breastfeeding.

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Misused of medicines in urban and festive areas

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Introduction: An evaluation of the nature of the psychoactive substances misused in the urban and festive areas seems to be necessary in order to alert the emergency unit system. This evaluation, made by the CEIP (Centre d'Evaluation et d'Information sur la pharmacodépendance) system, allows to identity numerous medicines sold as ecstasy pills.

Methods: Samples (pills, powders, capsules, liquids…) were colleted by the SINTES device (Système d'Identification National des Toxiques et Substances) tool for the French monitoring centre for drugs and drug addiction (FMCDDA). A physical description and a photography were performed before analysis. Immunochemical researchs are practiced in order to find stupefying molecules as amphetamines, cocaine, cannabis and opiates. Complementary analysis with chromatography mass spectrometry techniques allows to identity the different components.

Results: Analysis lead to discover numerous molecules of medicine. Three main groups were present: first group with psychotropics, second group with an attractive logo, third group with substances used for dilution. The discovered psychotropics are mainly alimemazine, buprenorphine, venlafaxine ketamine, The specialities with attractive logos are various like buflomedil, chloroquine, betamethasone, floctafenine. The molecules used for dilution are chloroquine, lidocaïne. For some medicine, the galenic aspect varies, for example for chloroquine which is found as pills, but also as powder for used for diluting cocaïne.

Conclusion: This study allows to improve the knowledge about the misused medicine molecules, and to inform health professionals and authorities in order to propose an adaptation of the French law following each case and substance.

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Myocardial infarction and atrial fibrillation with lenalidomide: a case report

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Introduction: Multiple myeloma is a malignant hemopathy characterized by an invasion of clonal plasma cells in the bone marrow. Despite the progress made with autologous stem cells transplantation, the prognosis remains poor. The prognosis of this hemopathy has been recently improved with the development of new drugs. Lenalidomide is one of these new drugs. Lenalidomide has a particular status in France, as it is delivered within a 'temporary use authorization' procedure.

Methods: We report on the case of a 61-year-old man treated with lenalidomide for refractory multiple myeloma who developed an myocardial infarction with atrial fibrillation (AF).

Results: Multiple myeloma was diagnosed in 2002. From June 2002, different protocols were used without any marked efficacy. In July 2006, lenalidomide (25 mg/day 21 days/28 days) and dexamethasone (40 mg J1–J4) were prescribed. The patient had no cardiac history: ECG and echocardiography were normal. Creatinine clearance, thyroid function tests and serum electrolytes were normal. After 10 days of lenalidomide administration, the patient was hospitalised for thoracic pain with dyspnea and administration of lenalidomide was stopped. Myocardial infarction with atrial fibrillation (AF) was diagnosed. In September, lenalidomide was rechallenged because of the evolution of myeloma. After about 7 days of treatment, the patient had another FA episode. The administration of lenalidomide was definitively abandoned.

Conclusion: The responsibility of lenalidomide was retained in front of: (i) positive rechallenge in a patient with no cardiac history; (ii) case reports of cardiac adverse effects with thalidomide, a parent compound of lenalidomide. Dysrhythmia (essentially bradycardia), myocardial infarction and increase of QTc interval were reported in clinical trials.
Few cases of dysrhythmia have been reported with lenalidomide in the literature. Two cases of atrial fibrillation with lenalidomide 25 mg/day are registered in the French Pharmacovigilance database. So this drug shows a cardiac toxicity close to that of thalidomide.

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DRAMES: a database for identification of causes of drugs abusers deaths

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Introduction: DRAMES (Décès en Relation avec l'Abus de Médicaments et de Substances) database is a national collecting system of deaths related to psychoactive substances abuse. The observations are reported by 16 voluntary forensic analysts.

Methods: The cases of death with toxicological analysis results and which correspond to the A definition of drug-related deaths of the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) are included.

For each death, an anonymous form, approved by The Council of Forensic Science in 2001, is filled by the expert with: individual characteristics, history of abuse, circumstances of the fatal abuse, and toxicological analysis results (substance identification and blood level).

All forms are transmitted to the CEIP (Centre d'Evaluation et d'Information sur la Pharmacodépendance) in charge of the study.

Results: In 2005, 68 deaths were reported, 65 of them directly induced by the substances (for the three other cases: drowning, defenestration, accident in subway). 80% of men, average age of 31 years. Illicit drugs, mostly heroine and cocaine, were involved in the majority of the case series (48 of 65–73.5%). Opiates substitution treatments were at the origin of 10 deaths (eight cases with methadone). Other legal opiate medicines (codeine and morphine) induced 10% of the reported deaths. Polyintoxications (alcohol and/or cannabis found in one third of the case series) and associations with legal psychotropics (43%), mainly benzodiazepines, are frequent.

Conclusion: DRAMES is one of tools to evaluate the possibility of abuse of psychoactive substances. Its data are used by the French Ministries of heath and Interior. To improve the system, several possibilities are studied: inclusion of new forensic analysts in order to cover the whole of the French territory; collaboration with hospital analytical partners for collecting deaths occurring in their hospitals; and try to complete analytical data with autopsy reports.

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Hyponatraemia in an elderly patient treated with tramadol

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Introduction: Drugs are a common cause of electrolyte abnormalities. One of the commonest drug-induced electrolyte abnormalities is hyponatraemia.

Methods: We report on the case of a 87-year-old woman with hyponatraemia following short administration of tramadol. This patient was hospitalized for lumbalgia in Rheumatology Unit (8/11/2006). Medical history included hypertension (treated for years with spironolactone and altizide), osteoporosis and arthrosis. The treatment in hospital was:

- spironolactone (25 mg) + altizide (15 mg): one tablet/day,

- tramadol (37.5 mg) + paracetamol (325 mg): three tablets/day stopped on the 9/11/2006,

- nefopam: 3 times/day instead of tramadol + paracetamol thereafter.

Results: Routine blood tests included sodium 130 mmol/L, potassium 3.9 mmol/L, chloride 93 mmol/L creatinine clearance 61 mL/min (8/12/2006). On 12/11/2006, she was noted to be confused and blood tests were performed. The results were: sodium 98 mmol/L, potassium 3.3 mmol/L, chloride 69 mmol/L. She had a low serum osmolality of 217 mOsm/kg (normal range: 275–295 mOsm/kg) with normovolemia. Thyroid function tests and head CT scan were normal. Antihypertensive medication was discontinued on the 13/11/2006. Serum ADH was normal on 14/11/2006. Then serum sodium level gradually increased to 128 mmol/L within the following 10 days.

Treatment consisted on oral fluid deprivation and infusion of 500 ml of saline 0.9%/24 h.

Conclusion: Drug-induced syndrome of inappropriate antidiuretic hormone (SIADH) was suspected. Urinary tests were not performed, so SIADH could not be formally confirmed. But as hyponatraemia was improved after the drug-withdrawal, and rechallenge with spironolactone + altizide was negative we hypothesise that tramadol may have been directly involved in this patient's biochemical disorder or at least related to the combination of tramadol and spironolactone + altizide.

Two case-reports of hyponatraemia associated with tramadol have been reported in the literature and the WHO Adverse drug reactions database contained 18 reports of this adverse effect with tramadol. SIADH can be caused by a variety of drugs, particularly carbamazepine, selective serotonin reuptake inhibitors (SSRI) and phenothiazines. Tramadol is a synthetic opioid with serotonergic properties. Opioids can directly stimulate increased ADH secretion. Tramadol also produces an increased concentration of serotonin at the synaptic cleft. Therefore another possible mechanism for such an adverse effect may lie in the similarities of the pharmacology of tramadol and SSRI.

This case report underlines a potential new adverse effect of tramadol.

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Clopidogrel resistance is associated with increased risk of in-stent restenosis

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Introduction: In-stent restenosis (ISR) remains an important limitation after stent implantation, especially during the first 30 postprocedural days. Clopidogrel, in association with aspirin, is currently the drug of choice to prevent ISR. Recent studies have demonstrated distinct response variability and resistance to clopidogrel therapy based on *ex vivo* platelet function measurements. Our objective was to investigate if clopidogrel resistance is associated with a heightened risk of ISR. Moreover, we attempted to find the effect of cardiovascular risk factors or medication on occurrence of ISR.

Methods: A retrospective study was undertaken at Toulouse University Hospital (France) from 1st March 2004 to 31st May 2006. Patients were selected using hematology laboratory data, based on reduction of ADP-induced platelet aggregation (resistance test to clopidogrel). Medical records of all selected patients were then consulted. All patients undergoing a percutaneous coronary intervention (PCI) with stenting and a double antiplatelet therapy by clopidogrel and aspirin were selected. Then, the patients were classified into two groups according to occurrence of ISR after PCI. The two groups were compared by their responsiveness to clopidogrel, different cardiovascular risk factors, dosage of clopidogrel, drug therapy, length, type and number of stents. Student's t test and chi-squared test were used to assess differences between the two groups.

Results: Forty-one patients (age: 66.7 ± 11.8 years) with an ISR, 20.2 (±37.0) days after PCI (group I) vs. 40 patients (age: 69.5 ± 10.7 years) without any cardiovascular complications (group II) were included in the study. Twenty-six patients from group I were resistant to clopidogrel vs. Eight in group II ($P < 0.0001$). Group II received a higher dose of clopidogrel after PCI than group I (143 mg ± 30 vs. 90 mg ± 30, $P < 0.0001$). We did not find any significant difference between the two groups concerning length, type (drug-eluting stent or simple stent) or number of implanted stents, cardiovascular risk factors (cigarette smoking, diabetes, hypertension, dyslipidemia, family history and obesity) or drug therapy (statins, ACE inhibitors, beta-blockers, diuretics, etc).

Conclusion: Our data show that nonresponsiveness to clopidogrel was significantly more frequent in patients presenting an ISR post-PCI. Moreover, the patients without ISR post-PCI had a more elevated clopidogrel dosage which could reduce the risk of ISR. These data should be confirmed by other surveys in larger samples in order to define other parameters affecting post-PCI complications.

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Concept of a coordinated approach to optimize the spontaneous declarations of pharmacovigilance in hospital

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Introduction: The French pharmacovigilance system is based on spontaneous declarations by health professionals on the serious or unexpected adverse drug reactions. This is a professional duty. But a lot of studies highlight an important under notification.

Methods: An inventory of fixtures carried out in our establishment sheds an interesting light on three levels of difficulty: (i) doctors would like to receive conclusions from the Regional Center of Pharmacovigilance on their observations; (ii) nurses who notice an adverse drug reaction don't always think of reporting it; and, (iii) pharmacy students (students in their 5th year practicing in clinical services and interns) who have to encourage doctors to report the adverse drug reactions, don't seem to know the exact process of notification. These findings lead us to brainstorming sessions, in collaboration with the Regional Center of Pharmacovigilance, to try to improve each one of the levels.

Results: In partnership with the Regional Center of Pharmacovigilance, we established a process that ensures that the notifications' conclusions from Regional Center of Pharmacovigilance are returned to the consultants. A flow chart was created and validated.

Moreover, a teaching and practical procedure was developed. It presents the principles on which the notification works and it is structured on ?QQOQCP' method (what, who, where, when, how, why). An annex reminds the theoretical concepts of imputability. The second attachment is a step by step guide to students on how to fill out the reporting form. This flow chart also appears in the appendix on procedure. Already, 10 students in their 5th year were able to put this procedure into practice and evaluate it. The average marks obtained are 15.5/20 for the utility and 16/20 for comprehensibility.

Finally, to make nurses aware of pharmacovigilance's declaration, this topic has been developed in our local information newspaper (intended for the nurses).

Conclusion: After validation by the pharmacists and the Regional Center of Pharmacovigilance, doctors were informed of the procedure's availability during hospital's drug committee. They are satisfied with the return of notification set up. And this procedure is now systematically given to the students in their 5th year when they begin training.

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Prenatal defects of topiramate and levetiracetam: a case report

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Introduction: Few data were available about the exposition during pregnancy to new antiepileptic drugs as topiramate and levetiracetam. Preclinical studies revealed a teratogenic effect of topiramate, but not for levetiracetam. The small number of human cases does not permit to conclude about a potential teratogenic risk. In lack of epidemiologic studies, it is important to report all new cases of defects, especially for drugs recently available on the market.

Methods: A child who was exposed to both drugs at the beginning of pregnancy presented severe malformations. He was born spontaneously at 35 weeks of gestation and died after 30 minutes of life.

Results: The mother, 42-years old, took levetiracetam and topiramate for pharmacoresistant epilepsia before conception. Dosage was decreased at week 21 of gestation and stopped at 33 weeks of gestation. No problem occurred at the beginning of the pregnancy. An amniocentesis was realized at week 12 of gestation because of the mother's age. The karyotype 46 XY was normal. On the occasion of the echography of third trimester, at week 32 of gestation, multiples diseases were

revealed concerning right lung, cardiac and renal organs, despite normal weight and measurements for his age. The post-mortem analysis revealed right diaphragmatic agenesia and right lung hypoplasia without cardiac disease.
Conclusion: This case suggested the implication of levetiracetam and topiramate in the onset of these malformations because no other etiology can be suspected. Malformations found in our case report were different from the ones reported in literature. As well, this kind of malformation is rarely reported in patients treated with antiepileptic drugs. We recommend that a follow-up should be carefully done in all pregnancies exposed to these drugs.

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Accidental child poisonings with cannabis derivatives reported to Lyon Poison Centre between 1999 and 2006
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Introduction: There has been an increasing trend in Cannabis use over the last decade. Therefore, the risk of acute exposure in young children following accidental ingestions should be considered with the intrusion of cannabis derivatives into the parental and domestic environment.
Methods: The present study is based on a retrospective analysis of all phone calls for child poisonings due to accidental cannabis ingestion received at Lyon Poison Centre between 1999 and 2006. The inclusion criteria were: age under 15 years, acute ingestion and accidental circumstances.
Results: Out of the 29 collected cases, 27 were eligible to analysis. The medium age was 19.4 months (8–60 months), with a slight female dominance (55%). Cannabis resin was the most frequently involved presentation. The symptoms mostly consisted of drowsiness and/or gait disturbance and/or hypotonia. In addition, mydriasis was noted in four children. Analytical screening for cannabinoids was not performed in all cases, but was positive in the 16 children for whom it was performed. Overall, the symptoms noted in this retrospective analysis were similar to those reported in the medical literature. More severe disorders, e.g. Marked CNS depression and bradypnoea have nonetheless been reported in some cases.
Conclusion: Cannabis derivatives are a potential, even though still uncommon cause of severe poisoning in toddlers. Suspicion must be raised in those children presenting with reduced consciousness of unknown cause, ataxia, mild mydriasis, bradypnea. An analytical screening is advisable to rule out other toxic agents.

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An automated method to eliminate bias induced by co-prescription in safety signal generation using spontaneous reporting database
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Introduction: Automated disproportionality analysis of spontaneous reporting is increasingly used routinely. It can generate a huge amount of signals that require highly time-consuming expert assessment, most signals finally appearing irrelevant. These false positive signals rely on several mechanisms among which drug co-prescription can lead to such false positive signals, as for gastro-intestinal bleeding where a signal can be generated for ascorbic acid, because of its frequent association to acetylsalicylic acid. We present here an automated method to minimize the number of signals generated by confounding due to co-prescription.
Methods: This automated method is based on a backward stepwise removal of all reports involving the drug leading to the most important signal for an event in the database, until no signal remains. Signals were identified using the case non-case approach, the most important being those with the highest ROR and an IC95% excluding 1. If for instance for gastro-intestinal bleeding, the highest signal in the database concerns NSAIDs, all reports with NSAIDs will be removed from the database, leading to the generation of a second database, in which the highest signal could concern acetylsalicylic acid. All reports concerning this drug will then be removed leading to the generation of a third database and so on. We tested this method for hepatitis over 16 years of spontaneous reporting in the French Pharmacovigilance database.
Results: In the whole database, we initially identified 20 signals for hepatitis NEC. The backward stepwise procedure successively removed signals for phenytoin, valproic acid, carbamazepine, amineptin, febarbamate, phenobarbital, metformin, pravastatin, dydrogesterone and dapsone. Signals that were initially generated in the whole database and that no longer appeared using this method concerned progabide, topiramate, clobazam, iron and vitamins, vigabatrin, clonazepam, lamotrigin, amphetamin, diazepam and buspirone.
Conclusion: This study demonstrates that the number of false positive signals generated by automated methods using spontaneous reporting databases can be highly reduced by methods based on the exploration of biases specific to pharmaco-epidemiology, such as channelling due to co-prescription.

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Drug interactions with cholinesterase inhibitors: an analysis of the French Pharmacovigilance database
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Introduction: Cholinesterase inhibitors (ChEIs) could be involved in several drug-drug interactions because of their complex pharmacodynamic or pharmacokinetic properties. We performed an analysis into the French Pharmacovigilance database (FPD) to identify case reports containing drug-drug interactions (DDIs) involving ChEIs (donepezil, galantamine or rivastigmine).
Methods: Spontaneous reportings recorded in the FPD concerning donepezil, galantamine or rivastigmine were reviewed by two clinical pharmacologists from Toulouse Regional Pharmacovigilance Center. Case reports containing DDIs were identified according to French National Formulary (Vidal), British National formulary (BNF) or their own judgment. Then, the responsibility of DDIs in the occurrence of adverse drug reactions (ADRs) registered into the FPD was evaluated. Finally, summary Characteristics of Products (SCP) of the different ChEIs in the two references (Vidal, BNF) were compared relating to their DDIs informativity.
Results: Among 1058 case reports involving ChEIs and registered in the FPD until the 31st March 2006, 376 (35.5%) contained at least one DDI line according to experts' judgment and 118 (11.2%) were the cause of ADRs. Most of the DDIs were due to pharmacodynamic interactions (247 cases, 65.7%). DDIs were found in 309 (29.2%) case reports according to Vidal and in 127 (12%) according to BNF. 88 (8.3%) ?serious' ADRs were related to DDIs (including seven deaths, mainly due to cardiovascular ADRs). Comparison of different SCPs showed that Vidal was more informative than BNF for all the ChEIs and that galantamine had the most complete data into the two used references.
Conclusion: Pharmacovigilance database could be used to investigate DDIs. They are frequent for ChEIs and occur in more than one third of cases. They led to ADRs approximately in one third of cases. Informativity of drug dictionaries largely differs, especially for DDIs between bradycardic drugs (digoxine, amiodarone…) and ChEIs.

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Hypersensitivity reaction to phenobarbital in a fat patient
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Introduction: Phenobarbital is an antiepileptic drug with a long half-life elimination. It is a lipophile drug that can be accumulated in panniculus adiposis. The occurrence of side effects was often related to over doses. The allergic reaction remained exceptional.
We report a case of hypersensitivity reaction induced by phenobarbital with slow amelioration (6 weeks) and lesions extension after drug withdrawal. The clinical evolution was confronted with determining of phenobarbital plasmatic level.
Methods: A 38-years old man, weighing 110 kg and heighing 1.8 m. Operated at January 2000 for a benign cerebral tumor. This patient had been treated from 01/15/2000 with phenobarbital 15 cg/day. Twenty–two days after treatment beginning, this patient developed a generalized erythematous maculopapular plates, as well as multiple vesicle and pustule associated with facial oedema, oral ulcers.
Results: Face to these clinical features, phenobarbital was withdrawn and replaced by phénytoïne since 02/09/2000. The patient has received also local symptomatic treatment. At 02/14/00, the patient didn't present any improvement and at this date, phenobarbital level was 9.62 mg/L. A week later (02/21/00) the pustulous lesions disappeared, erythema, pruritis and peri-orbital oedema were decreased and phenobarbital level was 5.5 mg/L. At 02/28/00 the patient came back with intensification of erythema and oedema, at this day phenobarbital level was 4.75 mg/L. At 03/06/00 the patient present a second episode of cutaneous lesion aggravation but at this time we could not determine phenobarbital plasmatic level because the patient refused the prelevements. At 03/27/00 lesions had completely disappeared and phenobarbital level was 0. Responsibility of phenobarbital was retained with I3B3 score.
Conclusion:
Phenobarbital responsibility was retained because:
phenobarbital was the alone drug taked before appearance of lesions evolution was very suggestive: slowly evolution can be explained by the long half-life elimination of phenobarbital and aggravation of lesions can be explained by, drug accumulation in panniculus adiposis favorised by patient corpulence, and release of this drug in plasma controlled by phenobarbital monitoring.

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Tipranavir French cohort ATU: safety results from 295 patients
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Introduction: Tipranavir co-administered with low dose of ritonavir (TPV/r) is a new generation protease inhibitor (PI) with a potent activity against multiple PIs resistant HIV-1 viruses. TPV/r is indicated for combination antiretroviral treatment of HIV-1 infected adult patients with evidence of viral replication who are highly treatment experienced or have HIV-1 strains resistant to multiple PIs. The objectives of TPV French cohort ATU were to provide access to TPV/r and evaluate in current practice the safety of TPV/r.
Methods: From 18 July 2005 to 15 March 2006, 295 triple class experienced patients were entered in the cohort ATU and were treated with TPV/r 500/200 mg bid. According to the cohort ATU protocol and AFSSaPS requirements, only adverse drug reactions had to be reported by physicians. However, all reported adverse events (AEs) regardless of physician's causal relationship and seriousness were recorded into the company drug safety database and presented in this analysis. Procedure of notification, data gathering, evaluation and validation of the pharmacovigilance cases were performed in collaboration with the Regional Pharmacovigilance Centre in charge of the cohort ATU.
Results: A total of 295 patients entered in the cohort ATU. 84.4% were male; mean age was 44.4 years (18–70). At baseline mean viral load was 4.01 $\log_{10}$ copies/mL and mean CD4 cell count was 219 cells/mm^3. TPV/r was combined with 1, 2, 3 or more than 3 antiretroviral drugs for 6.1%, 29.8%, 44.1% and 16.3% of patients respectively. T-20 was combined with TPV/r for 72.2% of patients. The mean treatment duration was 104.5 days (2–234). The estimated overall exposure was 84.4 patients-years. 44/295 patients (14.9%) experienced at least one AE while treated with TPV/r. 18/295 patients (6.1%) developed serious AEs (including four deaths not related to TPV/r by the physician), 20 (6.8%) discontinued TPV/r due to AE and only 22 (7.5%) experienced at least one AE considered as related to TPV/r. The overall incidence of SAEs was 21.3 per 100 patients exposure years (PEY) which was consistent with RESIST data (23.9 per 100 PEY). Five patients (1.7%) experienced nine serious AEs (SAEs) considered as related to TPV/r which were all expected and included in the following System Organ Classes: hepatobiliary disorders (one hepatitis, two hepatic cytolyses, two biological hepatitis), investigations (AST and ALT increased), general disorders (asthenia) and gastrointestinal disorders (nausea). Eight related SAEs were resolved (one unreported outcome). No new safety issue was identified and no intracranial haemorrhage or other serious bleedings were reported.

Conclusion: During the French TPV cohort ATU, analysis of collected AEs did not reveal any new safety aspects or any change in frequency or severity of the already known AEs for TPV/r confirming the favourable safety profile of TPV/r in current practice.

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Hemorrhagic colitis (HC) and isotretinoin: analysis of cases reported in French Pharmacovigilance database

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Introduction: Isotretinoin is a vitamin A synthetic analog used for treatment of severe and resistant forms of acne. Main adverse reactions are known and well described in the literature but although they are reported on drug legal mentions, isotretinoin-induced HC represents few reports in literature (1,2).
Methods: We analyzed isotretinoin-induced HC notified to the FPD from 1990 to 2006.
Results: Eight cases of HC were notified to FPD. Gender ratio was 1. Mean age was 24 ± 5 years, mean dose was 35 mg/day. 62.5% of HC appeared during treatment (mean duration: 108 days) and 37.5% after treatment withdrawal (mean: 23 months). Endoscopic diagnosis conducted in four cases revealed inflammatory colitis. Recovery was observed in five patients. Rechallenge was positive in two cases. Reaction leaded to hospitalization for three patients and in persistent or significant disability for two patients. Seven patients received only isotretinoin therapy when symptoms occurred. Drug causality relationship was possible in four cases, probable in three cases and likely in one case.
Conclusion: Cases of HC induced by isotretinoin are scarce but the seriousness of symptoms must be better known. Mechanisms proposed are: disturbance of epithelial cell maturation, alterations of glycoprotein metabolism compromising the colonic mucosal integrity and induction of killer T-cell activity (1–2). Isotretinoin appears to act as a trigger for HC and inflammatory bowel disease (IBD) in general and have been reported to aggravate preexisting IBD; so careful consideration about isotretinoin treatment should be made in patients at higher risk for IBD.

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Drug-induced dementia: a case/non-case study in the french pharmacovigilance database

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Introduction: The increased incidence of dementia with age and the population aging makes this group of diseases a major problem for public health. Among known etiologies of dementia, drug therapy are under considered. This study investigated the relationship between exposure to drug therapy and dementia using French Pharmacovigilance database.
Methods: We use the case/non-case methodology. Cases were reports of dementia, worsened dementia or demential syndrome (which included cognitive impairment) recorded in the French Pharmacovigilance database and non-case were all reports of reactions other than these being studied. The studied period was from January 1985 to December 2005. We calculated the odds ratio (OR) of reports associated with dementia for drugs for which three cases of dementia were reported. Data of literature were analysed for the drugs which are associated with an increased risk of dementia and if mention of dementia was present in the summaries of product characteristics (SPC).
Results: Among the 263 962 adverse drug reactions recorded in the database, 79 (0.03%) were dementia. Median age was 66 (range 2–91) with 62% being 65 and more. Dementia were associated with the use of psychotropic drugs for the majority of cases and the risk of dementia was significantly increased with valproate (OR 14.9 [9.5–23.3]), trihexiphenidyle (OR 9.2 [3.6–23.8]), zolpidem (OR 5.9 [2.7–13.2]), lorazepam (OR 4.7 [1.9–11.7]), acepromet azin (OR 7.9 [3.4–18.4]), lithium (OR 8.9 [3.4-23.2]) and buprenorphin (OR 8.9 [3.4–23.2]). Antidepressants selective serotonin re-uptake inhibitors, hypnotics, phenothiazin neuroleptics and benzodiazepin anxiolytics were also associated with an increase risk of dementia like the following non psychotropic drugs: interferon alfa 2B (OR 9.2 [3.5–23.7]), vancomycin (OR 3.8 [1.3–11.1]), and allopurinol (OR 3.6 [1.4–9.3]).
Conclusion: The adverse effect ?dementia' is only mentioned in the SPC of valproate but not in the SPC of other psychotropic drugs and some non psychotropic drug which psychiatric effects were described in the litterature. Whatever the etiology, dementia result in neurotransmission deficit. Cholinergic hypothesis was retained, but disturbance of gabaergic pathways was also evoked. Drugs with anticholinergic effects like tricyclic antidepressant and anti Parkinsonian drugs, benzodiazepin and neuroleptics drugs and with hyponatremic effect like diuretics and selective serotonin re-uptake inhibitors may generate troubles like dementia. Drug etiology for dementia was not considered in the aging population. We encourage health professionals to notify all psychiatric effects.

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Necrotizing colitis during neuroleptic treatment: analysis of the French Pharmacovigilance database and review of the litterature

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Introduction: Necrotizing colitis is a pathology based on micro-vascular disruption of intestinal wall leading often to death, despite emergency surgery. Only about 20 cases of necrotizing colitis associated with neuroleptic treatment are reported in the litterature. Phenothiazines as chlorpromazine have been most involved suggesting that the risk is related in great part to anticholinergic activity. Recently, cases with clozapine, atypical antipsychotic with anticholinergic properties, have been published.
Methods: We analysed all cases of necrotizing colitis during neuroleptic therapy notified to the French Pharmacovigilance database (FPD). Patient's demographic data, neuroleptic therapy, dosage, associated treatments and outcome were recorded.
Results: Twenty cases of necrotizing colitis during neuroleptic therapy have been notified in the FPD: nine men, 10 women, and a new born. The mean's patient age was 37 ± 14 years-old. Clinical symptoms were poor including abdominal pain, nausea, diarrhea, and were not correlated with the seriousness of evolution. Surgery was effective in 70% of patients. Death is reported in 45% of cases. 40% of patients had sequelae, 5% totally recovered, the outcome being unknown in 10%. Thirty per cent of patients had a neuroleptic monotherapy, 50% bitherapy, 15% tritherapy, 5% quadritherapy. Sixty-five per cent of patients had at least one anticholinergic associated medication such as antihistaminic, antidepressant, and anti Parkinsonian. Phenothiazines are involved in 65% of cases: cyamemazine (seven cases), levomepromazine (five cases), both of them (one case). Death occurred in patients receiving neuroleptic monotherapy with potent anticholinergic activity or association of several weaker anticholinergic drugs. High dosage of neuroleptic was associated with fatal outcome.
Conclusion: Necrotizing colitis during neuroleptic treatment is rare but mortality is high in spite of non specific symptoms. Physicians should be aware in patients receiving neuroleptics with anticholinergic properties in association with other anticholinergic drugs. Close monitoring of digestive functions seems required with decrease the number of associated anticholinergic drugs and administration of laxative therapy.

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Does drugs without adverse effects really exist?

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Introduction: when an adverse effect is suspected by a general practitioner, it is usual even before calling the nearer pharmacovigilance center to open and read his physician desk reference, in France 'le dictionnaire vidal'; if the adverse effect he is seeking for is not mentioned in the summary of product characteristics, he should undertake some expensive exploration and, anyway, he should not dechallenge the drug.
It seems of interest looking for drugs without adverse reaction rubric fulfilled in the 'dictionnaire VIDAL'. Surprisingly we find 228 drugs without adverse effects it is noticeable that for some drugs with several presentations, the lack was not always concerning all presentations.
Methods: So it seems interesting to classify these products according to présentation and use.
Topical drugs: 24
Phytotherapy: 52
Homeopatic medicines: 25
Vitamines: 19
Eye drops: 15
Venotonic drugs: 7
Oligoelements: 16
Allergens: 1.
Results: Among concerned drugs it is interesting to remark that some excipients we find in these drugs should explain some adverse effects. Paraben were found in 31 cases and it is said that they are involved in 1% of drug allergy. Ethanol was found in 30 cases, which is not a problem in normal use
but accidental children ingestion should be worrying. Aroma: 24 case, synthetic aromas being a complex melting of products and we found vanillin, a known allergen, in four cases. Dyes: 18 sunset yellow, eight food yellow 13, two food red 7, two food red 14
Terpenoids: 15 Preservatives: 16 especially with eye drop.
Conclusion: In the French pharmacovigilance data bank we find with these 228 drugs 354 cases where the drug implicated was used alone by the patient when the adverse effect take place. It seems interesting to details these adverse effects and perhaps to complete, for some drugs, the summary of product characteristics to avoid some surprise among general practitioner and even patients.

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Children Born from HIV + mothers at Clermont-Ferrand and St-Etienne: 2006–2006 Clinical Survey

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Introduction: Health condition of children born from HIV positive mothers is poorly known and that's why we made this study to harmonize these children's follow-up and care management adapted to eventual anomalies screening. To ameliorate this follow-up we thought of describing clinical condition of children, searching, before they reached 2 years of age, a mitochondrial pauci-symptomatic biologic anomaly and describing, after 2 years old, their learning their familial and social environment.
Methods: To reach these objectives, it was decided to identify couple of mother-child and get parents' consent, to collect maternal characteristics and the ones from the children from birth to their actual age and to make a clinical examination adapted to the age.
Results: Eighty-two couples of mother-child were identified. Thirty-six mothers accepted to participate in the study. For 36 children (with 28 of them being more than 2-years old), a clinical consultation or a dossier could be done. Thirty of the above-mentioned cases are not infected by HIV because they received ARV during foetal and neonatal period. Among the six other cases that are HIV positive, only one was treated during foetal period. However all of these six patients now benefit from treatment. No major trouble was detected during this study.
Conclusion: Available data on the long-term follow-up of children born from HIV positive mother are scarce. Despite the little number of subjects in this study, this study allowed to better figure out the evolution of this population.

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Spontaneous reporting of Adverse Drug Reaction in an Emergency Department from a French University Hospital

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Introduction: Incidence of hospital admissions related to adverse drug reactions (ADRs) was between 2.4–6.5%. ADRs were described as one of the most frequent drug-related visits to emergency departments (ED). The aim of the study was to evaluate the characteristics of ADRs among ED in a French University Hospital.
Methods: The study was performed between May 2005 and June 2006. Spontaneous ADRs reports from the ED of Toulouse Purpan University Hospital, were reviewed. For each reported ADR, we noted information about patient (age, gender), characteristics of ADR(s) and drug exposure (suspected and concomitantly used drugs).
Results: A total of 76 spontaneous ADRs reports was evaluated. Mean age of patients admitted in ED for ADRs was 67 ± 20 years (range: 15–92 years, male/female ratio: 0.68). ADRs were more frequent in old patients (>70 years: 62% of the patients). Patients aged from 40 to 49 years ranked in second position (16%). Drugs more frequently imputed were nervous system (33%) followed by anticoagulant (24%) and cardiovascular (18%) drugs. The system organ the most often reported was nervous system disorders (25%), followed by blood (20%) and cardiovascular (17%) disorders. In patients aged 80 years or more (30% of the patients), 22% of ADRs were related to anticholinesterasic agents, ranking in the second position just after anticoagulants. In patients aged between 40 to 49 years, male/female ratio was 5. Drug more frequently imputed was antibiotics and the most frequent ADR was gastro-intestinal disorders.
Conclusion: The study, performed in an ED, clearly corroborates that ADRs are more common in older patients. Its also underlines the risk related to anticholinesterasic agents use in older patients and indicates that men aged 40–49 years represents a population at risk for ADRs. Monitoring of ADRs reports from ED could provide useful information about pharmacoepidemiology of outpatients ADRs.

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Mesalazine-induced oligoamnios: first case report

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Introduction: To date, in the literature, more than 300 cases of women treated during pregnancy with mesalazine are available without any increase of congenital abnormalities risk or foetal toxicity. We describe the first case report of oligoamnios related to mesalazine.
Methods: A 29-year-old woman began a second pregnancy on September 2005 the 12th under orally mesalazine, 1 g/day. This only treatment was performed since 2001, for an inflammatory colitis. Her first pregnancy has been complicated by a pregnant diabetes mellitus and the first baby was born healthy whereas mesalazine exposure. At the end of the 5th month of pregnancy, the foetal ultrasonography revealed an oligoamnios. At 28 weeks' gestation, the oligoamnios is confirmed at 35 mm. The fetus was eutrophic and foetal kidneys were without abnormality. The cardiac rhythm was correct. Umbilical, cerebral and uterine arteries Doppler were normal. A premature membrane rupture was excluded. As no maternal pathology (infection, arterial hypertension) was diagnosed, the responsibility of mesalazine was suspected in the oligoamnios occurrence. Mesalazine was discontinued at 29 weeks' gestation. The oligoamnios still present until the 34 weeks' gestation, improved spontaneouly and the foetal ultrasonography performed at 36 weeks' gestation shows normal amniotic fluid level (100 mm). The patient underwent a caesarean at 39 weeks' gestation due to a breech position. The female baby was eutrophic, and her kidney ultrasonography was normal.
Results: Mesalazine (5 amino salicylic acid) is the active moiety of salazosulfapyridine. Mesalazine crosses the placenta to the fetus. Foetal: maternal serum ratio is around 1, despite the administration route. In toxicological studies, mesalazine is associated with glomerular and tubular nephrotoxicity in rats with a dose dependant occurrence. Colombel et al[1] reported a renal insufficiency in a neonate boy after prenatal exposure to mesalazine (2–4 g/day between the 3rd and the 5th month of pregnancy). The baby's renal biopsy showed tubular atrophy and interstitial fibrosis with glomerular microcysts in the adjacent cortex. The creatinine clearance was 52 mL/min at 6 months.
Conclusion: Due to the absence of other materno-foetal explanation, and regarding the positive dechallenge, the responsibility of mesalazine should be considered in the occurrence of this oligoamnios. The mechanism of this toxicity may be explained by a foetal nephrotoxicity by inhibition of prostaglandin synthesis.

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Outcome of pregnancies after suicide attempt involving medicines

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Introduction: Only a few publications have examined the possible embryofetal consequences of acute drug poisonings during pregnancy. Our objective was to describe a cohort of women who attempted suicide during pregnancy and to analyse the outcome of pregnancy.
Methods: Requests received from January 1994 to December 2004 by both Lyon Poison and Pharmacovigilance Centres were analysed. Information on the patient, details on acute drug exposure, and management of poisoning were collected at the time of the initial inquiry. The outcome of pregnancy was the primary objective and was assessed prospectively.
Results: A cohort of 237 pregnant women who attempted suicide with medicines was identified. Their mean age was 27.1 ± 6.6 years. A previous episode of attempted suicide was noted in 25% of these patients and four attempted suicide twice during the same pregnancy. Acute poisoning occurred during the first 4 weeks of pregnancy in 13 cases (5.5%), during organogenesis in 105 (44.5%) and later during pregnancy in 119 (50.2%). One single medicine was ingested in 137 cases (58%). Drugs of the central nervous system were the most commonly used (294 out of 405 acute exposures), in particular benzodiazepines (26%) and antidepressants (12%). Concomitant acute alcohol exposure was identified in 10% of patients. Severe immediate complications were noted in 19 patients but no women died. The outcome of 144 pregnancies was documented and included 25 elective abortions (17.4%, 95% CI: 11.6–24.6), three medical abortions (one malformation, two psychiatric indications), eight miscarriages (5.6%, 95% CI: 2.4–10.7%), and 108 deliveries (111 live-birth infants with four major malformations). The mean gestational age at birth was 38.8 weeks and 16 neonates were premature (14.4%, 95% CI: 8.5–22.4%). A major congenital malformation was identified in five of 112 newborns or examinable fetuses (4.5%, 95% CI: 1.5–10.1), but a temporal relationship with acute drug poisoning was likely in only two cases, namely Pierre Robin syndrome after bromazepam exposure at week 5 and polymalformation syndrome after mirtazapine, prazepam and zuclopenthixol exposure at week 4.7.
Conclusion: Although the sample size is small with a high rate of lost to follow-up (39%), this study provides some reassuring data and suggests that acute drug poisoning during pregnancy does not seem to carry an increased risk of major malformations. In addition, the spontaneous abortion rate was not increased as compared to the usual rate in the general population.

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Analysis of the incidence of medicinal anaphylactic shocks among adults: survey at the Regional University Hospital Center from 1999 to 2005

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Introduction: Anaphylactic shock is the most severe form of anaphylaxis. It occurs rapidly in the organism after introduction of the allergen and can compromise vital prognosis.
It is recommended to proceed to an allergology consultation from 6 to 8 weeks after the shock. As well, fibrinolysin (tryptase) dosing is recommended between 1 to 6 hours after the onset of symptoms. It is mandatory to declare all anaphylactic shock cases to the Regional Centre of Pharmacovigilance. This study focuses on the application or not of these recommendations, as well as the analysis of these shocks (aetiology, severity).
Methods: The method consists in gathering information on the 67 anaphylactic shocks of the Regional University Hospital Centre and to analyse it. A consultation of the pneumo-allergology dossiers and of those from the fibrinolysin dosing laboratory is done afterwards. A search in the declared observations of the Regional Centre of Pharmacovigilance is carried out.
Results: We found 62 cases of anaphylactic shocks of medicinal or latex origin among the sixty-seven cases. Seventeen of these 67 above-mentioned cases were seen in allergology at the University Hospital. Only 44% of the 32 peranaesthesic reactions of the survey were explored in allergo-anaesthesia consultation. On the 62 medicinal shocks, only eight of them were declared in Pharmacovigilance (33%). Among the cases who were explored in hospital allergology consultation, only three of them were declared at the Regional Centre of Pharmacovigilance. Fibrinolysin (tryptase) dosing was done in all patients. However, 10 dosages were done in an appropriate delay, where five of them were done in an unknown delay. The curares are the most frequently found substances among the ones where responsibility was shown by cutaneous tests or IgE specific tests in allergology. Nevertheless, they account for only seven cases. However, among suspected but not established diagnosis, the most incriminated substances are the ones used in anaesthesia and latex, a total of 20 of the 46 cases in this category.
Conclusion: Conclusion: non-respect of these rules of practice about allergology consultation and pharmacovigilance declaration appears to be very frequent. We can hope this survey will reach to a dialogue between the different healths professionals implied. It would be more than advisable to put in place important measures as an eventual interdisciplinary protocol and to study its impact.

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Effect of abnormal Savda munziq on the morphological and ultrastructural changes of target organs in an abnormal savda syndrome animal model

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Introduction: Abnormal savda munziq (ASM) is a preparation used in traditional Uyghur medicine to counteract the effect of abnormal savda, a condition associated with chronic diseases such as NID diabetes, hypertension or asthma. This study was designed to observe the effect of ASM on the morphological and ultrastructural changes of target organs in an abnormal savda syndrome animal model.
Methods: Male ICR rats were fed with cold-food diet under cool dry environment, stimulated with electric foot shocks to establish abnormal savda syndrome animal model of Uighur medicine. Four groups were studied: one control and three treated with increasing doses of ASM (2.53 g/kg, 5.06 g/kg, 10.12 g/kg) during the whole procedure.
The animals' brains, cardiac muscles and livers were observed in optic and transmission electron microscopy to document histological and ultrastructural changes.
Results: Microscopic examination of the control group showed adipose cells infiltration and interstitial angiectasis of cardiac muscles; in the liver there was liver cells regeneration, point necrosis and liver tissue inflammatory infiltration. There was severe concomitant lesions of the cell organelles on electron microscopy. These alterations were dose-dependently opposed by ASM.
Conclusion: This model of traditional Uyghur medicine syndrome of abnormal savda is associated with morphological and ultrastructural damage of the target organs. ASM, which is traditionally used in man to treat abnormal savda, will in animals dose-dependently oppose these structural damages.

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Cardiac tolerance towards antipsychotic combinations

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Introduction: Sudden deaths have been reported in patients receiving psychotropic drug since the early 1960s. Some antipsychotic drugs seem to be more frequently associated with a prolongation of the QT interval. Increasing length of the QT interval can degenerate into torsade de pointesand the combination of drugs

increase the risk of sudden death. As precaution, in 2001, based on literature, the French agency for the sanitary security of health products (AFSSAPS) published a list of twelve antipsychotics (chlorpromazine, cyamémazine, lévomépromazine, thioridazine, triflupérazine, amisulpride, sulpiride, sultopride, tiapride, dropéridol, halopéridol and pimozide) whose association is ill advised (and contraindicated with sultopride). Today this list has still not been valided by tests. The aim of this work is to study the effects of the combinations of antipsychotics which are inadvisable according to the AFSSAPS recommendations, based on the QT interval in a population of hospitalised patients.
Methods: The study was carried out during a 16-month period among patients hospitalised in a psychiatric unit. The QT interval on the patients' ECG was measured manually by a single investigator, then corrected by Bazett's formula. We divided patients into three groups: patients with inadvisable combination of antipsychotic, patients with combination that was not inadvisable, patients who had not received any antipsychotic (control group). We compared in an univariate study QTc values distributions of the three groups by using the non parametric test of Mann–Whitney. Then, different factors involved in the lengthening of QTc where included in a multivariate analyse.
Results: A total of 350 patients were included in this prospective study. The non-parametric analysis by the Mann–Whitney's method confirms that patients who received an inadvisable combination ($n = 14$) presented QTc intervals longer than those who received a combination that was not inadvisable ($n = 133$, $P = 0.006$). The latter patients presented longer QT intervals than those who had not received any antipsychotic ($n = 111$, $P = 0.025$). The multivariate linear regression showed the independence of the different factors recordered in the study: sex ($P = 0.002$), age ($P = 0.001$), tricyclic treatment ($P = 0.022$), combination of antipsychotic treatment inadvisable (0.000) and combination of antipsychotic treatment not inadvisable ($P = 0.000$).
Conclusion: Our study confirms by experimentation the conclusions reached by the AFSSAPS based on literature. However, the impact of recent antipsychotic drugs is badly known: The pharmacovigilance statements are thus essential for the AFSSAPS.

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Assessment of adverse drug events with Alzheimer's disease medications: information about notifications addressed to Pharmacovigilance Centres from the North West of France
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Introduction: Ageing of the population is growing with increasing incidence of dementia, especially Alzheimer's disease. We have nowadays four drugs indicated in this disease: donepezil, rivastigmine and galantamine which are three acetyl-cholinesterase inhibitors and memantine, an inhibitor of N-methyl D-aspartate receptors.
Methods: We collected in the french pharmacovigilance database all notifications addressed to four Regional Pharmacovigilance Centres in the North West of France and involving these drugs as they have been introduced in the French market. Data about patients (age, body weight, number of pathologies, number of daily drugs) and about the suspected drugs, the most serious side effects, outcomes and imputability according to the french method were analyzed.
Results: We found 71 case reports of adverse drugs events involving one of the Alzheimer's disease medication. The most involved drug was donepezil (41), with less notifications concerning rivastigmine (16), galantamine (8) and memantine (6). Sex ratio was 1.09. The mean results were: age of patients 79 ± 7.2 years, body weight 63.7 ± 11.8 kg, number of daily drugs 4.45 ± 2.04. Most of patients suffered from polypathology (57.7%). More than a half side effects were serious (62%), with one third concerning cardiac function, and one third central nervous system. There was a high score of imputability in 31% of cases. There has been a good outcome in most of cases (80%), but two patients had sequels and three died. In 21% of cases, the adverse events occurred at the beginning of the treatment or when increasing the dosage. A drug–drug interaction was present in nine notifications, with clinical relevance only in one. Age of patients who develop a side effect at the beginning of treatment was higher but not significative (83.8 ± 6.5 vs. 78.4 ± 6.8, $P = 0.07$), and number of daily drugs wasn't higher in this group (3.75 ± 1.26 vs. 5.25 ± 2.12).
Conclusion: These results point out the risk of serious adverse drug events with acetyl-cholinesterase inhibitors, especially cardiac and neuralgic. Adverse drug events may have heavy consequences in old people with dementia, so it would be very useful to study their incidence confronted with drug benefit in long term prescription.

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Serious adverse events occurring during clinical trials: a French university hospital promotor experience
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Introduction: The objective of the present study was to describe serious adverse events (SAEs) notified during clinical trails to an university hospital, as promotor of clinical studies.
Methods: From January to November 2006, 473 events were addressed by clinical investigators to the monitoring unit of the institutional sponsor. All events were assessed by the monitor and classified as serious/not serious and related/unrelated to the study. Only study-related events were notified to the competent authority. Some SAEs were also reviewed by a security committee (when available).
Results: Nineteen clinical studies were concerned, mainly involving drugs (63%) or medical device (16%). Thirty-two percents were oncology studies and 2/3 multicenter trials. Clinical studies were focused on haematological (26%), cardiological (21%) or neurological (16%) drugs. Five per cent of events were not considered as ?serious' and 43% of events came from university hospital Toulouse. Mean number of SAEs was 26 ± 28 events per study (median: 4). Mean delay between occurrence and sponsor declaration was 182 ± 332 days (median: 47 days). Despite the lack of informations in many reports, 30% of events were "possibly related" to the clinical research. Other events were related to the progression of the disease or intercurrent events. Evolution of these events was favourable outcome (57%), ?not resolved' (16%), death (15%).
Conclusion: This study underlines the importance of SAEs for an institutional promotor of clinical studies. The new legislation context of clinical trials recalls the necessity for institutional promotors to involve regional pharmacovigilance centers in this increasing activity.

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The use of levonorgestrel, an emergency contraception method, investigated in a community pharmacy
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Introduction: All contraception methods available need a planned use before sexual intercourse and entail a risk of failure, linked to either the method itself or its inaccurate application. Emergency contraception appears as an emergency alternative treatment when a risk of unwanted pregnancy is suspected. Using reliable information technology, it was observed an abnormally high dispensation of the "morning after pill" in our city. From this observation, it seemed to us interesting to determine the profile of users and to understand the reasons why it is used so frequently.
Methods: From 28 March to 15 May 2006, 2 types of questionnaires were suggested in a 24 h pharmacy. The first, 12 questions-long, was filled by 73 users of the method. The second questionnaire, 14 questions-long, was filled by 27 any non-user to whom this product was dispensed (sexual partner, friend, family member, neighbor, colleague).The survey was given by the same pharmacist.
Results: During the survey period, 100% of emergency contraception seekers came in the pharmacy without prescription. We observed a peak of dispensing on the Sunday morning. Twenty percents of users were minors and 60% were 20–39 years. The main sources of information about the availability of the contraceptive method were the person's circle and the media. Fifty-four percents of users never used this method before, but two patients, 18 and 20 years old, had used it respectively 8 and 9 times. No one asked for it more than 72 hours before a sexual intercourse. In 55% of cases, the need of the method was due to condom accident, in 13% to oral contraceptive oversight and 28% of people had used no contraception at all. If 93% of seekers were aware that there is a risk of pregnancy despite the use of the method, 17/73 of users didn't know that they had to envisage a pregnancy test after 5 days of lateness of their menstruations. Seven percents of seekers thought that the method protected the user from all sexually transmitted diseases. The women's information main sources were their surroundings, media or school, but not health professionals. Obviously, they are still poorly informed.
Conclusion: Levonorgestrel seems to become a common emergency contraception more than an emergency contraception to use only in special cases. 31% of women seem to not consider any other contraception method. It is good news that women now use the method, the transmission of information for a more rationale use should be emphasized by health professionals when prescribing or delivering the medicine.

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Preventability of adverse drug reaction in elderly with dementia
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Introduction: Iatrogenic pathology is known to be frequent in elderly, preventable adverse drug reactions become currently a priority of public health. In France, dementia concerns 855 000 persons and we wanted to determine the compliance to treatment, the incidence of adverse drug reactions which lead to hospitalisation and could be avoided in a sample of elderly people with cognitive impairment.
Methods: We included in our cohort study all elderly patients aged 80 years and over with cognitive impairment, evaluated by mini mental status, who were recently hospitalised in a short geriatric unit of a French university hospital. Inclusion began in September 2003 and lasted 6 months. Medical files and interview of the patient and his family allowed to collect retrospectively data about medical history (including instrumental activities of daily living interview), drug prescription and real intake, with assistance or not. Preventability was evaluated with a score of four items: not preventable, possible, plausible and likely.
Results: Eighty-two patients, mostly women (73%), aged 87 (±4.9) years with cognitive impairment (mini mental status = 15.8 ± 5.7) were included; insrumental activities of daily Living showed that 94% were dependent elderly, with visual or hearing problems for more than 60% of them; in average, they have prescription of 5 ± 1.5 drugs, the most frequent drugs used were psychotropic drugs (31%), oral anticoagulants (24%), analgesics (17%) and diuretics (17%).
Thirty-three per cent had no assistance method to avoid errors in drug intake, leading to 37 cases of observance error, among them, 13 were directly responsible of hospitalisation. Forgetting and intentional non-compliance were the most frequent type of error (38% and 19%).
Thirty cases of adverse drug reactions included mostly neuropsychiatric events (26%), falls (20%), metabolic disease (18%), haemorrhage (17%) and cardiovascular effects (11%).
Finally, 37% of hospitalisation was related to adverse drug reactions, among them, more than half could be preventable mostly by improving compliance. The underestimation of the cognitive impairment, leading to an inadequate help, is the major preventable factor highlighted by this study.
Conclusion: Thus, a preventable risk of adverse drug reaction exists in elderly with dementia related to under-evaluation of dementia. Therefore, search for cognitive impairment is of great importance, to provide some support to compensate for it and provide an adequate drug utilisation in this population.

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Is the A/B/C/D alphabetical classification of adverse drug reactions easily applicable?
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Introduction: Various mechanistic classifications of adverse drug reactions (ADRs) have been proposed in literature. Nevertheless, the original A/B classification suggested by Rawlins and Thompson has persisted. Type A reactions

result from "an exaggeration of a drug's normal pharmacological actions when given in the usual therapeutic dose, normally dose dependent". Type B reactions represent "aberrant effects that are not expected from the known pharmacological actions of a drug". This classification has been extended to other alphabetical types, including type C ("continuous") and type D ("delayed"). The purpose of our study was to apply the A/B/C/D alphabetical classification to ADRs notified to the Toulouse's Pharmacovigilance centre and then discuss the difficulties to apply it.
Methods: We chose ADRs notified during 1 year (2005) and, among these, we selected those with causality assessment ≥I2 "plausible". For each pair "drug/ADR", we analysed drug information (pharmacological class, respect of dose or not, causality), ADR information (class-organ, type A/B/C/D, time to onset, outcome, seriousness, type "expected/unexpected"). If more than one drug was suspected, we counted all pairs "drug/ADR" implicated. We present here the descriptive analysis of these cases.
Results: Among the first ADRs notified in 2005, 67 were classified in type A, 24 in type B and two in type C. Type A reactions concerned hematological, cutaneous and neurological ADRs. All were "expected" (i.e. Labelled in summary of characteristics of the product). Dose was correct in all cases but one. The most frequently implicated drugs were those acting on central nervous system (analgesics, antipsychotics) and anticoagulants. Type B reactions concerned cutaneous, hematological and neurosensorial ADRs. Sixty per cent were "unexpected". Dose was correct in all cases. Drugs involved in type B reactions were drugs acting on central nervous system and anti-infectious. Two ADRs have been classed in type C.
Conclusion: This study shows that most of the ADRs (around 80%) reported to a CRPV are from type A. However, we met with some difficulties to class ADRs in type B. Type B reactions are defined as "not to be expected from the known pharmacological action of a drug". Thus, we were inclined to class all "unknown mechanism" into this category, that represents a bias.

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Adverse effects of bortezomib: a prospective survey
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Introduction: Bortezomib is a specific reversible proteasome inhibitor approved for the treatment of relapsed or refractory multiple myeloma. Each course of treatment consists of four intravenous administrations (1.3 mg/m^2) every 3 weeks. Clinical trials suggested that bortezomib is generally well tolerated with mild-to-moderate gastrointestinal toxicity as the most frequent, and peripheral sensory neuropathy as the most clinically relevant adverse effects. As little data is available in routine clinical practice, our aim was to assess the adverse effects of bortezomib in a cohort of 14 consecutive patients.
Methods: Clinically relevant adverse effects associated with bortezomib administration were prospectively collected using a standard data form containing specified criteria and filled out systematically after each course of bortezomib. Patients were followed up from January to August 2006. Adverse effects were assessed for the time to onset, possible risk factors, severity, outcome, and impact on disease management.
Results: Over the 7-month period, 14 patients received a total of 61 courses (225 injections) of bortezomib, i.e. 4.36 courses per patient. A total of 48 serious adverse effects (21.3% of injections) were reported in 11 patients. Seven patients experienced neurotoxic symptoms that represented 12 episodes of adverse effects (25%). Other adverse manifestations consisted of gastro-intestinal toxicities (17%), febrile reactions (17%), cutaneous reactions (one generalised toxidermia and two episodes of rash and erythema) (8%), asthenia (6%), hematological disorders (anemia in two and severe thrombocytopenia requiring platelet transfusion in one) (6%), and two episodes each of limb oedema and psychiatric manifestations (agitation and insomnia). An infectious episode was also noticed in six patients. Neurotoxic symptoms occurred generally at night and required dose reduction (1 mg/m^2) for the subsequent courses in seven patients and symptomatic clonazepam treatment in 1. Bortezomib was finally definitely discontinued in two patients because of peripheral neurotoxicity. In addition, further bortezomib administration was contra-indicated in two other patients who had experienced serious diarrhoea and severe toxidermia.
Conclusion: In this prospective real-life survey, serious adverse effects were observed in 11 of 14 patients (79%), an incidence higher than in clinical trials. In particular, dosage reduction (50%) and definitive discontinuation of treatment (29%) were more frequently required than expected. Finally, further surveillance of neurotoxicity is needed to determine the most appropriate management that will allow continuation of treatment.

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Adverse effects of rituximab: a prospective survey
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Introduction: In clinical trials in patients with B-cell lymphoma, rituximab was considered to be well tolerated compared to standard chemotherapy. The most frequent adverse effects consisted of infusion-related reactions in approximately 10% of patients. However, limited data is still available on rituximab safety in common clinical practice. We report our experience from a cohort of 103 consecutive patients treated for various lymphoid malignancies.
Methods: Prospective study of clinically relevant adverse effects associated with rituximab infusion using a standard data sheet containing 39 pre-specified criteria, and filled out systematically after each treatment course. Patients were included from November 2004 to February 2005. Follow-up was completed until discharge, or resolution of the adverse effect. All reported adverse effects were assessed for the time to onset, possible risk factors, severity, outcome, and impact on disease management.
Results: During the 4-month period, 103 patients (59 males, mean age: 66 years) received a total of 335 infusions. Thirty-four patients received rituximab alone (104 infusions) and 23 had a previous history of cardiovascular disorders. No clinically relevant adverse effects were reported in 80 patients (78%). The 23 remaining patients experienced a total of 58 adverse effects (2.5 adverse effects per patient) that occurred during the first course of rituximab in one half of them. Mild-to-moderate infusion reactions that usually occurred shortly after starting the infusion were noticed in 14 patients for a total of 23 episodes (40% of adverse effects). One of these patients experienced a moderate cytokine-release syndrome. Other adverse effects included 11 (19%) cardiovascular complications, 6 (10%) hematological disorders, 6 (10%) gastro-intestinal disorders, 6 (10%) dermatological lesions and 6 (10%) respiratory disorders with one case of interstitial pneumonitis. These adverse effects required a reduction in infusion rate or transient treatment discontinuation in 18 patients (78%), symptomatic treatment in 15, and hospitalization in only 3. Further rituximab administration was contra-indicated in one patient who experienced a severe hypotensive episode (50/30 mmHg) during the fourth infusion.
Conclusion: This systematic survey of rituximab-associated adverse effects in common clinical practice seems to confirm the safety profile described in clinical trials. Particular attention to interstitial pneumonitis and cytokine-release syndrome remains mandatory.

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Adverse drug reactions: a survey in liver-transplant patients
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Introduction: Immunosuppressive drugs given after organ transplantation can lead to serious Adverse Drug Reactions (ADRs). New immunosuppressive drugs, such as tacrolimus and sirolimus, are marketed as an alternative to ciclosporine. Upon the request of liver-transplant (LT) patients, we have undertaken an observational study to collect ADRs from LT patients in collaboration with the Multiorgan Transplant Unit (MTU) team of Toulouse University Hospital.
Methods: LT patients from Midi-Pyrenees area, who attended the MTU clinic, received a mailed questionnaire (after giving their informed consent) that included questions about age, cause of transplantation, length of transplantation, use of drugs, concomitant disease, and ADRs. At the following consultation, they completed the questionnaire with the help, if necessary, of our team. Clinical or biological data were also obtained from medical files.
Results: A total of 118 patients [sex ratio (M/F): 1.81, mean age 54.6 years ±9.6, (27–72)], participated to this survey. The mean transplant duration was 56.5 months (±52.4). The main causes of liver transplantation were alcoholic cirrhosis (31.6%), hepatitis C virus (HCV) infection (31.6%), hemochromatosis (8.8%), primary biliary cirrhosis (PBC) (7%), hepatitis B virus (HBV) infection (7%) or tumors (7%). Immunosuppressive drugs used were tacrolimus (79.3%), ciclosporine (18.1%) or sirolimus (2.6%). Patients were also exposed to other drugs (mean value 5.9 ± 2.8 drugs) as follows: prednisone (48.3%), mycophenolate mofetil (20.3%), recombinant erythropoietin (15.3%), alpha interferon (9.3%), G-CSF (5.9%), azathioprine (5.9%) proton pomp inhibitors (30.5%) and statin (28.8%). A total of 1.389 [neurological (30.1%), cutaneous (13.2%), hematological (12.4%), osteomuscular (11.2%), gastrointestinal (10.1%), cardiovascular (8.2%), and various (14.9%)] ADRs were collected. Multivariate analysis showed that tacrolimus-induced tremor occurred more in patients recently transplanted (<18 months) related to high doses of tacrolimus ($P < 0.0004$). Association of immunosuppressive drugs to statin favored the occurrence of osteo-muscular ADRs [$OR = 4.2$ (1.2–14.5)]. Corticotherapy did not increase significantly the occurrence of osteoporosis. The risk of arterial hypertension, hirsutism and gingival hypertrophy was significantly higher ($P < 0.0001$) in patients exposed to ciclosporine more than 79 months.
Conclusion: This study underlines the interest of ADRs' self reporting by patients in order to improve the collect of ADRs non serious but nevertheless affecting patients' quality of life.

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Vitamin K epoxide reductase (VKORC1) genetic polymorphism is associated to venous thromboembolism: results from the EDITH study
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Introduction: The vitamin K epoxide reductase (VKOR) recycles endogenous vitamin K, a co-factor for vitamin K-dependent coagulation factors synthesis. Common polymorphisms in *VKORC1*, the gene coding for VKOR have been found to affect vitamin K antagonists dose response, and recently to confer an increased risk of vascular diseases in a Chinese population. The aim of this study was to evaluate the association between the *VKORC1* 1173C>T polymorphism and venous thromboembolism.
Methods: We report the results of a case–control study designed to evaluate interactions between acquired and inherited risk factors of VTE. We studied 439 cases hospitalised with a first venous thromboembolic event not related to a major acquired risk factor for VTE and 439 controls matched for gender and age. *VKORC1* 1173C>T polymorphism was selected for genotyping as the tagging single nucleotide polymorphism (SNP) for previously identified *VKORC1* haplotypes.
Results: The relationship between VTE and *VKORC1* 1173C>T polymorphism was consistent with a recessive model. Odds ratio (95% CI) was 0.62 (0.41–0.94) for TT genotype compared to CT/CC genotypes suggesting *VKORC1* TT genotype was associated with lower risk of VTE. Adjustment on cardiovascular diseases, body mass index, Factor V and Factor II gene mutations did not alter the results.
Conclusion: In this case-control study, *VKORC1* TT genotype is associated with a significant lower risk of venous thromboembolism. Other investigations are required to precise underlying mechanisms.

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Hormone therapy and risk of venous thromboembolism among postmenopausal women. Impact of cytochrome P450 3A5 genetic polymorphism. The Esther Study
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Introduction: Oral estrogen use increases the risk of venous thromboembolism (VTE). Transdermal estrogen may be safe with respect to thrombotic risk. Expression of the human cytochrome P450 3A5 (CYP3A5) is implicated in the

hepatic catabolism of steroidal hormones, especially estrogen. Subjects with the CYP3A5*1/*1 and CYP3A5*1/*3 genotypes express the enzyme. Therefore, we investigated the impact of the CYP3A5 genetic polymorphism on the association between hormone therapy by route of estrogen administration and VTE risk.
Methods: We performed a multicenter case-control study of VTE among postmenopausal women who were enrolled in 1999 through 2006 at eight clinical centers and in the general population, in France. CYP3A5 genotype was successfully evaluated in 193 consecutive cases with a first documented episode of idiopathic VTE and in 534 controls. Relative risks were estimated by odds ratios (OR) and 95% confidence intervals (CI).
Results: The allele frequency of CYP3A5*1 was 9% and 10% among cases and controls, respectively (*OR* = 0.9; 95% CI: 0.6–1.5). Oral but not transdermal estrogen increased VTE risk compared with non-users (*OR* = 3.8; 95% CI: 2.3–6.5 and *OR* = 1.1; 95% CI: 0.7–1.7, respectively). Compared with non-users, OR for VTE in current users of oral estrogen was 3.1 (95% CI: 1.7–5.4) among patient without CYP3A5*1 allele and 22.1 (95% CI: 3.7–133.2) among patients who expressed the enzyme (test for interaction of CYP3A5*1 allele and oral estrogen on VTE risk was significant, $P = 0.04$). By contrast, there was no significant interaction of CYP3A5*1 allele and transdermal estrogen use on VTE risk.
Conclusion: Women with CYP3A5*1 allele using oral estrogen can define a subgroup at high VTE risk. If confirmed, these findings could benefit women in the management of their menopausal symptoms with respect to the VTE risk associated with oral estrogen.

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Prognostic value of ribavirin AUC 0–12 h after the first dose in genotype 1 chronic hepatitis C patients given ribavirin and peginterferon alpha-2a (40 kDa)
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Introduction: Current models used to predict response to treatment with pegylated interferon plus ribavirin are generally based on viral decline during the first 12 weeks of therapy. However, the rapid virological response (RVR) at week 4 has a great positive predictive value of sustained virological response. The aim of this work was to study the relationships between ribavirin exposure (i.e. Area under the ribavirin concentration-time curve –AUC-) after the first dose and RVR in patients with genotype 1 HCV (characterized by a poor virological response).
Methods: A bicenter clinical trial (CINAM) was conducted in 28 naive patients infected with genotype 1 HCV who were administered peginterferon alfa-2a (40 kD) 180 µg/week and ribavirin with dose adjusted on body weight (<75 kg, 1000 mg/day, >75 kg 1200 mg/day). The database analyzed here consisted of 28 full ribavirin plasma concentration profiles. Ribavirin was determined in plasma using LC-MS/MS and RVR was defined as a virological load decline >2$\log_{10}$ over the first 4 weeks of therapy (Real-time PCR assay, HCV Amplipreptm Taqman).
Results: High inter-patient ribavirin AUC 0–12 h variability was found (range 1266–6916 µg/h/L), independent of the dose or dose/kg body weight. Fifteen RVR were observed. The mean AUC 0–12 h on day 1 was significantly higher in responders than in non-responders (4341 ± 1378 vs. 3025 ± 1030 µg/h/L, $P = 0.016$) The relationship between AUC 0–12 h and RVR was also analyzed with receiver operating characteristic (ROC) curves. The ROC area was 0.758 (CI 95% 0.556–0.900, $P = 0.0058$). A ribavirin AUC 0–12 h ≥ 3328 µg/h/L identified the patients with RVR with a sensitivity of 72% and a specificity of 80%.
Conclusion: This analysis suggests that ribavirin AUC 0–12 h after the first dose is a good predictor of RVR, but further studies including more patients and taking into account sustained virological response (W72) are needed to confirm these first results.

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Influence of cytochrome P450 2C19 and 3A4 gene polymorphisms on clopidogrel responsiveness in healthy subjects
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Introduction: Clopidogrel has become one of the first-line treatment for the prevention of atherothrombotic events in cardiovascular diseases. However, the efficacy of clopidogrel to inhibit ADP-induced platelet aggregation shows marked inter-individual variability and patients with lower response to clopidogrel are at risk for thrombotic complications. Clopidogrel is a pro-drug requiring a metabolic activation which is mainly mediated by cytochrome P450 isoenzymes (CYP). Recently, two common polymorphisms in genes encoding for the CYP2C19 (G681A, allele*2) and CYP3A4 (IVS10 + 12 G>A) have been reported to contribute to clopidogrel response variability.
Methods: We examined the influence of these polymorphisms and quantify their impact on clopidogrel responsiveness in 94 healthy subjects. All subjects participated in a randomized cross-over study where they received a 1-week course of aspirin 100 mg/day followed ($n = 45$) or preceded ($n = 49$) by a 1-week course of clopidogrel (300 mg on the first day, then 75 mg/day). The two treatments were separated by a 2-week wash-out period. ADP-induced (20 µmol/l) optical agregometry was performed at baseline and after each treatment period.
Results: After the 1-week course of clopidogrel, carriers ($n = 26$) of the CYP2C19*2 allele had a lower response to clopidogrel compared to non carriers ($n = 68$): the median reduction in ADP-induced platelet aggregation relative to baseline was 51.5% (interquartile range (IR): 28.4–65.9) in carriers vs. 63.2% (IR: 39.1–81.6) in non carriers of the CYP2C19*2 allele ($P < 0.001$). There was no subject homozygous for the mutated allele (*2/*2). The proportion of carriers of the CYP2C19*2 allele fell gradually across the quartiles of clopidogrel responsiveness, from 47.8% in quartile 1 (the poorest responders, $n = 23$) to 30.4% ($n = 23$), 25.0% ($n = 24$) and 8.3% ($n = 24$) in quartiles 2 through 4 ($P = 0.025$).
The observed difference remained significant after adjustment for age, platelet count, hematocrit, collagen lag time, and the fibrinogen and von Willebrand levels in a multivariate linear regression analysis. Carriers of the CYP2C19*2 allele were significantly more prone to be low responders to clopidogrel (odds ratio (OR): 3.0, CI: 1.0–8.7, $P = 0.048$). Such analysis showed however that the presence of the CYP2C19*2 allele accounted only for 10% of the global variability in clopidogrel responsiveness.
The presence of the CYP3A4 (IVS10+ 12A) allele did not influence the clopidogrel response. None of the studied polymorphism influenced aspirin responsiveness.
Conclusion: The present study replicates the CYP2C19*2 allele influence on clopidogrel responsiveness in a large population of healthy subjects and now urges investigations in the therapeutic setting.

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Isoniazid acetylation status in Tunisian people: phenotypic and genotypic aspects
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Introduction: As tuberculosis remains a relatively frequent disease, isoniazid is still widely prescribed. INH is metabolised mainly in the liver through acetylation by N-acetyl trasferase 2 (NAT2). The ability of the NAT2 to acetylate INH is subject to ethnicity and geographic location-dependent polymorphism of NAT2 gene. The present work aims to assess in a sample of Tunisian people the acetylation status by studying the phenotype and genotype of NAT2.
Methods: It was a prospective study performed in the pharmacology laboratory of the faculty of medicine of monastir. Blood from 68 patients with tuberculosis was used. After overnight fasting each patient received 5 mg/kg of INH as a single oral dose. After 3 hours, venous blood was collected and INH plasma concentration (C3) was measured by spectrophotometry. Therapeutic range of C3 was 1–2 µg/ml. The inactivation index I_3 was established according to Vivien equation: $I_3 = (C3 + 0.6)/D$ (D: INH dose).The phenotype was considered as slow if I3 > 0.65 and fast if I3 < 0.65. Recommended dose was calculated as follows: RD = (1.4 + 0.6)/I3. The most common known polymorphisms of NAT2 gene were identified from leukocyte DNA by real time-polymerase chain reaction (RT-PCR) and restriction fragment length polymorphism using the restriction endonucleotidases KpnI, TaqI, DdeI and BamhI.
Results: Sixty-eight patients (41 males and 27 females) were included in our study, they were aged between 7 and 88 years. C3 averaged 1.68 mg/l. According to I3, 79.4 and 20.6% were fast and slow acetylators respectively. I3 values were not correlated to neither age nor sex. Genotyping of NAT2 were performed in only 40 patients (12 were slow and 28 were fast acetylators as determined by I3). Five allele variants and nine different genotypes were determined. The commonest alleles were found to be NAT2*5B, NAT2*4, NAT2*6A (38.6, 30 and 10% respectively) and the most commonest genotypes were NAT2*4/5B, NAT2*5B/5B, NAT2*4/4. Genotyping of NAT2 categorized three groups of acetylators: seven patients (11.8%) were fast, 13 (32.5%) were slow and 20 (50%) were intermediate acetylators. Among the 12 slow acetylators 1 only subject is genetically found to be intermediate, however among the 28 fast acetylators, 20 were genetically intermediate.
Conclusion: Our study demonstrates, in a sample of Tunisian people, that intermediate acetylation status is predominant and shows that genotyping seems to be more relevant than phenotyping in determining acetylation status as it was difficult to discern intermediate from fast acetylator.

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MTX co-administration significantly increases ACR50 response to adalimumab regardless of multidrug resistance gene and methylenetetrahydrofolate reductase gene polymorphisms in RA patients from the ReAct study

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Introduction: The objective of this study was to determine whether *MDR1* and *MTHFR* gene polymorphisms are genetic predictors of response to adalimumab in rheumatoid arthritis (RA) in patients co-treated or not with methotrexate.

Methods: This ancillary study from the ReAct protocol included a large cohort of Caucasian patients with RA (n = 382) treated with adalimumab plus MTX (n = 186) or without MTX (n = 196). The primary outcome was ≥50% improvement in disease activity according to the core criteria of the American College of Rheumatology (ACR50) after 12 weeks of treatment. Patients were genotyped for *MDR1-C3435T* (n = 380), *MDR1-G2677T/A* (n = 377), *MTHFR C677T* (n = 380) and *MTHFR A1298C* (n = 380). For each gene, alleles and genotypes were tested for association with ACR50 response at week 12. MDR1 and MTHFR gene haplotypes were also examined for association with response to treatment after haplotype constructions using PHASE program. Univariate chi-square tests and multivariate logistic regression analyses were conducted for the entire study population and for the subgroups of patients with and without concomitant MTX.

Results: The profile of clinical response of the 382 patients included in this pharmacogenomic study was the same as that of the entire ReAct population (6610 patients). A total of 152 patients (40%) were ACR50 responders at week 12. As determined by univariate logistic regression, the probability of achieving an ACR50 response after 12 weeks of treatment was increased in the adalimumab plus MTX subgroup [P = 0.005-OR 1.76 (95% IC 1.13–2.73)]. Response to adalimumab was similar regardless of *MDR1-G2677T/A* (G/G, 40%; G/T + T/A 42%; T/T + A/A 46%) or *MDR1-C3435T* polymorphism (C/C, 38%; C/T 40%; T/T 45%).

For both *MTHFR* polymorphisms, the ACR50 pattern of response was also similar regardless of genotype: *MTHFR* C677T (C/C, 37%; C/T, 42%; T/T, 47%) and *MTHFR* A1298C (A/A, 41%; A/C, 41%; C/C, 34%).

Similarly, none of the reconstructed haplotypes (*MDR1*) were associated with ACR50 response.

Conclusion: This pharmacogenomic study is remarkable because of the size of the population as well as the quality of the clinical data recorded within the ReAct study. It provides robust data indicating that neither MTHFR nor MDR-1 gene polymorphisms in patients co-treated or not with MTX are predictive of response to adalimumab. These polymorphisms cannot account for the significantly higher rate of response observed when adalimumab therapy is with MTX.

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The cytochrome P450 (CYP) 2C9/2C19 but not the ABCB1 genetic polymorphism predicts liver CYP3A4 induction by phenytoin

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Introduction: We aimed to characterize liver CYP3A4 induction by phenytoin, a widely prescribed anticonvulsivant metabolized by the polymorphically expressed CYP2C9 and CYP2C19 and transported by P-glycoprotein encoded by the ABCB1 gene in extensive (EMs) and poor metabolizers (PMs) for CYP2C9 and CYP2C19, as well as the role of the ABCB1 genetic polymorphism in exon 26 (C3435T).

Methods: Eighteen healthy male volunteers (nine homozygous CC and nine homozygous TT) received 7-days treatment with 5 mg/kg bid of phenytoin. CYP2C9 and CYP2C19 genotyping for allelic variants *****2 and *****3 were performed using TaqMan allelic discrimination assay. Residual phenytoin plasma concentrations at steady state were determined at day 7 (D7) by fluorescence polarization immunoassay analyser. Liver CYP3A4 activity was evaluated using the ^{14}C-N-methyl erythromycin breath test (ERMBT) at baseline (D1), day 5 (D5) and day 7 (D7).

Results: Phenytoin concentrations were 9 ± 3 mg/L, 15 ± 3 mg/L and 26 ± 0.6 mg/L in homozygous EMs (*****1/*****1, n = 7), heterozygous EMs (*****1/*****2, *****1/*****3, n = 9) and PMs (*****2/*****2, *****3*****3, n = 2) for CYP2C9 or CYP2C19, respectively (P = 0.002, Kruskall Wallis test). Phenytoin significantly increased the ERMBT by 91.2% ± 43.6%, as compared to baseline (P < 0.001, paired Wilcoxon test). No difference between the ERMBT at D5 and D7 was observed. Divided into tertiles according to the phenytoin plasma levels (lower < 10 mg/L, middle 11–15 mg/L and upper tertile >16 mg/L), the maximum liver CYP3A4 activity was significantly different between lower and middle tertiles (3.5 ± 0.5% vs. 4.5 ± 1.0%, respectively, P = 0.02) and between lower and upper tertiles (3.7 ± 0.5% vs. 4.6 ± 0.7%, respectively, P = 0.05). The mean ERMBT results also differed between homozygous EMs and heterozygous EMs/PMs for CYP2C9 and CYP2C19 (3.5 ± 0.4% vs. 4.2 ± 0.8%, P = 0.03). The ABCB1 polymorphism had no influence on the extent of liver CYP3A4 induction.

Conclusion: Combined CYP2C9 and CYP2C19 genetic polymorphisms predicted liver CYP3A4 induction by phenytoin in healthy volunteers and may be used to anticipate the extent of drug-drug interaction occurring in patients treated with phenytoin.

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The cytochrome P450 3A5 genotype but not the ABCB1 genetic polymorphism predicts the daily dose of tacrolimus in renal transplantation

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Introduction: Tacrolimus (TAC) is a commonly used immunosuppressor for the prophylaxis of allograft rejection after organ transplantation. Therapeutic drug monitoring of TAC has been recommended due to its narrow therapeutic index and the important interindividual and intraindividual variability of its pharmacokinetics. The objective of this study was to investigate the relationship between tacrolimus (TAC) exposure and *CYP3A5 (Intron 3, G6986A)* and *MDR1* exon 26 *(C3435T)* genetic polymorphisms in renal transplantation.

Methods: We report the results of an ongoing pharmacogenetic study from the transgene study, a cohort of renal graft recipients from 1996 and 2006. Seventy-one patients, who were immediately treated with tacrolimus were included and genotyped for *CYP3A5* and *MDR1* single nucleotide polymorphisms. Genotypes were correlated to TAC concentration/dose ratio and daily dose requirement at 6 months and 1 year post transplantation.

Results: The *CYP3A5******1/*****1, *****1/*****3 or *****3/*****3 genotype was detected in five (7%), 15 (21%) and 51 homozygous (72%) of the 71 recipients. At 6 months post transplantation, TAC concentration/dose ratio was significantly lower in patients homozygous and heterozygous for the wild type (CYP3A5*****1) allele compared to CYP3A5 *****3/*****3 carriers (P = 0.0053). The median daily doses of TAC per body weight of TAC to obtain the target trough concentration were respectively at 6 months: 0.200, 0.165 and 0.100 mg/kg/d for *CYP3A5******1/*****1, *CYP3A5* *****1/*****3 and *CYP3A5* *****3/*****3 genotype (P = 0.0049). Similar results were obtained at 1 year post transplantation (P = 0.0068). MDR1 C3435T polymorphism was not related to the concentration/dose ratio and TAC daily dose requirement.

Conclusion: CYP3A5 genotype significantly influences tacrolimus metabolism after renal transplantation. Unlike MDR1 C3435T polymorphism, CYP3A5 (intron 3) genetic polymorphism appeared in our study to affect daily dose of tacrolimus. Screening for this single nucleotide polymorphism before initiation of tacrolimus therapy might be helpful for the selection of adequate initial daily dose and to reach the desired immunosuppression.

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Study of the G-protein beta 3 subunit gene (C825T) polymorphism and olanzapine or risperidone – related weight gain in patients with schizophrenia

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Introduction: Weight gain is a frequent adverse effect of psychotropic drugs, particularly atypical antipsychotics. It represents a major public health problem as it interferes with treatment observance and could participate to the cardiovascular mortality associated with psychiatric disease. No genetic predisposing factor has been yet associated with this adverse effect. C825T polymorphism of GNB 3, coding the beta 3 subunit of G proteins, was associated with obesity and, recently, with weight gain in a clozapine-treated Chinese population.

The purpose of this investigation was to examine association between C825T polymorphism of GNB 3 and olanzapine or risperidone weight gain treatment in a Caucasian schizophrenic population.

Methods: We conducted a pharmacogenetic association study to examine GNB 3 genotypes relation with olanzapine or risperidone induced – weight gain in a Caucasian population. Ninety-two schizophrenic patients treated with olanzapine or rispéridone were included and subsequently genotyped for this investigation. Most of these patients were hospitalized. Body weight was monitored before treatment initiation and after 6 weeks of olanzapine or risperidone treatment.

Results: Ninety-two patients were genotyped and the number (%) of subjects in each genotype group was CC = 36 (39%), CT = 47 (51%), and TT = 9 (10%) (T allelic frequency = 30.4%, no significant deviation from Hardy–Weinberg equilibrium). No statistically significant association was found between GNB 3 genotypes and absolute weight change (P = 0.13) or relative weight change (CC = 3.9 ± 4.6%, CT = 2.6 ± 3.6% and TT = 7.7 ± 9.4%, P = 0.13). Concordantly, GNB 3 genotypes did not influence body mass index (P = 0.21) (Kruskal-Wallis). Furthermore, no statistically significant difference in weight gain existed between subjects with a common allele and homozygous variant group (P = 0.50) (ANOVA). However we did observe a trend suggesting a potential relationship between the TT genotype and weight gain that warrant further investigation.

Conclusion: Preliminary results showed no statistical relationship between the C825T polymorphism and olanzapine or risperidone – related weight gain in Caucasian schizophrenic patients but clearly deserve additional investigations with larger sample size and longer observation period to determine if the T allele is a predictor of weight-related morbidity in schizophrenic patients treated with atypical antipsychotics.

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MDR1 C1236T, G2677T and C3435T genetic polymorphisms do not affect either response to lopinavir/ritonavir treatment or metabolic complications in HIV-1 infected antiretroviral experienced patients

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Introduction: P-glycoprotein, the gene product of MDR1, acts as an ATP-dependent efflux pump that exports a broad variety of drugs including HIV-1 protease inhibitors. Reports in the literature have provided conflicting results about the influence of MDR1 polymorphisms on the antiretroviral treatment effectiveness. The objective of the study was to investigate *in vivo* the impact of MDR1 polymorphisms on the metabolic complications and the response to a lopinavir/ritonavir (LPV/r).

Methods: LPV through concentrations were measured by high-pressure liquid chromatography with mass spectrometry detection at month 6 after the first administration of LPV/r. The changes in metabolic markers after LPV/r initiation were assessed for glucose, triglycerides, total cholesterol, liver enzymes levels at baseline and months 6. CD4 cell count and plasma HIV-1 RNA load were determined at each follow-up visit. We assigned *MDR1* 1236, 2677 and 3435 genotypes by real-time PCR allelic discrimination, and analysed their influence on pharmacokinetic parameters, immuno-virological outcome, metabolic markers using the non-parametric Kruskal-Wallis test.

Results: One hundred and ninety-two patients were enrolled in the study. The distributions of the *MDR1* C1236T, G2677T and C3435T polymorphisms were respectively: 37.5% C/C, 13.5% T/T, 49% C/T; 41.7% G/G, 14.1% T/T, 44.3% G/T;

28.1% C/C, 24% T/T, 47.9% C/T. Pharmacokinetic, immuno-virological parameters as well as metabolic markers were not significantly different with respect to the MDR1 1236, 3435 and 2677 genotypes (all P values >0.05).
Conclusion: Results suggested that MDR1 C1236T, G2677T and C3435T polymorphisms did not significantly affect either metabolic complications or response to a LPV/r-containing HAART.

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A 12 nucleotide insertion polymorphism in the human α2B-adrenergic receptor gene promoter that is linked to the glutamic acid deletion in the coding region impairs transcriptional activity
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Introduction: The α2B-adrenoceptor is expressed in blood vessels, and is responsible for the transient vasoconstriction following α2-agonist administration. Previous studies have demonstrated the existence of a common insertion/deletion polymorphism (+901 Ins/Del) in the coding region of the human α2B-adrenergic receptor gene (ADRA2B) that affects receptor function and is associated with an increased risk for cardiovascular events. This polymorphism was found to be linked with a G/C single nucleotide polymorphism at position −98 (numbered relative to the start codon). The present study examined the significance of a newly identified 12 nucleotide deletion/insertion polymorphism in the 5'-flanking region of the ADRA2B gene (−4825 del/ins) on promoter activity.
Methods: Subjects were genotyped by PCR/restriction enzyme digestion performed on genomic DNA extracted from blood samples. The possible functional significance of the promoter polymorphism was evaluated by transient transfection of luciferase reporter gene constructs into three unrelated cell lines (HEK 293, BHK-21 and HeLa).
Results: Genotyping of 71 unrelated Finnish individuals showed complete linkage between the −4825 del/ins and +901 Ins/Del polymorphisms. Reporter gene assays indicated that the −4825 ins/−98 C 5'-flanking region haplotype exhibits significantly reduced transcriptional activity when compared to the wild-type (−4825 del/ −98 G) haplotype.
Conclusion: Our findings show that the +901 Ins/Del and the variations in the 5' region of the human ADRA2B gene are linked, and that the promoter variations are associated with alteration of transcriptional activity *in vitro*. Further studies are warranted to elucidate the molecular mechanisms of this effect, and to delineate their possible impact on α2B-adrenoceptor expression *in vivo*.

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Anti-inflammatory effects of formoterol in rats after a single inhalation of nebulized cadmium
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Introduction: The aim of this study was to investigate the anti-inflammatory properties of formoterol, a long-acting β_2-adrenoceptor agonist, in a rat model of neutrophilic inflammation.
Methods: Animals were randomly assigned to a control group (n = 6) and a Cd group (n = 8) exposed to a nebulized vehicle (0.10% DMSO insaline) or to a 0.1% $CdCl_2$ solution during 60 min respectively. Three doses of formoterol (1 mg/30 ml, 2 mg/30 ml, 4 mg/30 ml of solution) were inhaled during 30 min just before cadmium or vehicle inhalation (n = 5–6/group). Specific airway resistance (sRaw) was measured using a double-chamber plethysmograph. Rats were then sacrificed and broncho-alveolar lavage fluid (BALF) was collected for determination of cell number, cytokines (IL-1β, TNF-α, GM-CSF) and total proteins concentrations. MMP-2 and MMP-9 activities were determined by gelatine zymography. Lung wet/dry (W/D) weight together with lung histology were also examined.
Results: Formoterol significantly attenuated scores of lung lesions associated with parenchyma inflammatory cell influx and congestion observed in Cd-group. It elicited a dose-dependent significant decrease in the following Cd-induced effects: total cell number ($8.6 \times 10^4 \pm 4.1$ (higher dose) vs. $23.2 \pm 7.7 \times 10^4$ cells/ml; $P < 0.001$), neutrophils ($2.3 \pm 0.6 \times 10^4$ vs. $11.7 \pm 4.0 \times 10^4$ cell/ml; $P < 0.001$) and macrophages counts ($2.5 \pm 1.0 \times 10^4$ vs. $9.2 \pm 3.2 \times 10^4$ cells/ml; $P < 0.01$), MMP-2 and MMP-9 activities (5.1 ± 1.2 vs. 6.6 ± 2.5; $P < 0.05$ and 0.5 ± 0.2 vs. 0.8 ± 0.2; $P < 0.001$), W/D weight (5.0 ± 0.3 vs. 5.4 ± 0.3; $P < 0.05$). The 57% increases in sRaw recorded after Cd administration was not influenced by formoterol. No significant change of cytokines was detected in any groups.
Conclusion: In conclusion, formoterol partially protects the lungs against the inflammatory effects of Cd by reducing lung parenchyma inflammatory cell infiltration but don't inhibit airway obstruction. This protective effect is associated with a moderate reduction of MMP-2 and MMP-9 activities known to play an important pro-inflammatory role in this model.
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The effect of abnormal savda munziq on morphological and ultra-structural changes of hypothalamus–pituitary–adrenal axis in an abnormal savda syndrome animal model
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Introduction: Abnormal Savda Munziq (ASM) is a preparation used in traditional Uighur medicine to counteract the effect of abnormal Savda, a condition associated with chronic diseases such as NID diabetes, hypertension or asthma. The objective of the study was to observe the effect of ASM on the morphological and ultrastructural changes of hypothalamus-pituitary-adrenal axis (HPAA) in an abnormal savda syndrome animal model.
Methods: Male ICR rats were fed with cold-food diet under cool dry environment, stimulated with electric foot shocks to establish abnormal savda syndrome animal model of Uighur medicine. Four groups were studied: one control, and three treated with increasing doses of ASM (2.53 g/kg, 5.06 g/kg, 10.12 g/kg) during the whole procedure. Changes of HPAA were observed by optic microscopy, and with transmission electron microscopy.
Results: Microscopic examination of the model group showed swelling of hypothalamic cells, interstitial angioectasis in the hypothalamus; there were moderate and severe pituitary corticotrope hyperplasia, moderate and severe adrenal cortex and medulla hyperplasia, especially zona reticularis cell of adrenal cortex. Electron microscopy showed concomitant ultrastructural changes. In the drug intervention groups, the morphological and ultra-structural changes were diminished in a dose-dependent manner.
Conclusion: This animal model of the abnormal Savda syndrome of traditional Uyghur medicine causes anatomical changes in the HPAA axis, that are opposed in a dose-dependent manner by ASM. This supports a relationship between the abnormal Savda syndrome and chronic stress on one hand, and a positive effect of ASM on this syndrome.

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Knee cartilage measurements during maturation and osteoarthritis follow-up using 7T high-resolution MRI: an *in vivo* study in the rat
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Introduction: High-field magnetic resonance imaging, which allows high-resolution imaging, is a powerful research tool to examine and visualize non-invasively hyaline cartilage of small joints. This experimental study was conducted (1) to assess *in vivo* age-related cartilage changes and (2) to follow osteoarthritis (OA) in rat knees from three dimensional (3D) datasets by using quantitative imaging, i.e. cartilage volume and thickness measurements, with histological confrontations.
Methods: Male Wistar rats were imaged *in vivo* at 7T using 3D high-resolution MRI (HR-MRI). 16 asymptomatic knees were scanned for the cartilage maturation process (four age-groups). 40 other rats underwent anterior cruciate ligament transection to generate experimental OA in their right knee (left knees being control). OA rats were imaged at Day 7, 14, 28, 42 and 56 after ACLT surgery. High resolution (51 x 51 x 94 μm^3 voxel size) 3D Gradient Echo with fat suppression sequences were performed with a specific home-made 2-elements network coil. Animals were then killed for histological study. Sagittal histological slices (5 µm, Haematoxylin-Eosin-Safran, Toluidine Blue, Sirius Red), corresponding to selected MRI weight-bearing areas, were achieved. For the two imaging modalities, femoral, medial and lateral tibial cartilages were manually segmented on a slice-by-slice basis. The resulting masks were then used to compute the knee cartilage volume and thickness (histology-MRI confrontations).
Results: In the normal maturation process, volumetric study of femorotibial cartilage revealed an initial volume increase (gp.1 vs. gp. 2, growing phase), followed by a progressive volume decrease (gp. 2–4, $P = 0.006$) inherent to the senescent phase. Additionally, correlation coefficients showed a significant correlation between MRI and histology for both femoral and tibial ($P \leq 0.02$) thickness. Histological scoring (synovitis, cartilage injuries) depicted significant differences between right (OA) and left (control) knees. OA MRI follow-up demonstrated high cartilage degeneration and volume loss in the femoral compartment, tibial plateaus being more affected by cartilage oedema.
Conclusion: HR-MRI at 7T allows *in vivo* analysis of age-related variations of rat knee cartilage. Quantitative HR-MRI values are compatible with expected thickness changes as depicted histologically. A significant correlation between the two methods is established. 7T 3D HR-MRI also demonstrates its acuity in experimental OA follow-up, thus offering a non-invasive, promising research tool for *in vivo* staging and monitoring therapy response in small rodents OA models.

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Anti-HIV effects of IFN-tau in human macrophages: involvment of cellular antiviral factors and IL-6
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Introduction: Tau interferon (IFN-τ) is a non-cytotoxic type I IFN responsible for maternal recognition of the fetus in ruminants. IFN-τ is developed as therapy of human diseases. IFN-τ was shown to inhibit human immunodeficiency virus (HIV) replication *in vitro* more strongly than human IFN-α, particularly in human monocyte-derived macrophages. In this study, the *in vitro* effects of IFN-τ were explored in human primary macrophages
Methods: Human peripheral blood mononuclear cells (PBMC) were isolated from healthy seronegative donors by ficoll-hypaque density gradient centrifugation. Monocytes were separated from PBMC by successive adherences and macrophages were obtained after 7 days of differentiation. Human macrophages are chronically treated by IFN-tau.
Results: IFN-τ efficiently inhibited the early steps of HIV biological cycle, decreasing intracellular HIV RNA and inhibiting the initiation of the reverse transcription of viral RNA into proviral DNA. Two mechanisms induced by IFN-τ treatment in macrophages may account for this inhibition: (i) the synthesis of the cellular antiviral factors such as 2', 5'-oligoadenylate synthetase/RNase L and MxA protein and (ii) an increased production of MIP-1α, MIP-1ß, and RANTES, which are natural ligands of CCR5, the main coreceptor of HIV on macrophages. These results suggested that IFN-τ induces the same antiviral pathways in macrophages as other type I IFNs, but without associated toxicity. In parallel, the immunomodulatory properties of IFN-τ were investigated in human macrophages. We found that IFN-τ increased the production of IL-10 and IL-6, but not of IL-1ß or TNF-α, in not infected and *in vitro* HIV-1/Ba-L-infected macrophages. We also found that the neutralization of IL-6 activity in the cell culture supernatants of IFN-τ-treated macrophages led to a decrease in the anti-retroviral effects of IFN-τ towards HIV RNA. Indeed, IL-6 co-operated with IFN-τ to decrease intracellular HIV RNA levels.
Conclusion: In conclusion, anti-HIV effects of IFN-τ are mediated by several modes of action, mediated either directly by IFN-τ or *via* other cytokines such as IL-6, also known to be induced by IFN-α.

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Involvement of ASICs in colonic hypersensitivity induced by butyrate in rats

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Introduction: The treatment of irritable bowel syndrome (IBS) characterized by colonic hypersensitivity, abdominal pain and bloating, is empirical and often poorly efficient. Based on the fact that butyrate concentrations are increased in IBS patients, we recently developed a rat noninflammatory model of chronic colonic hypersensitivity induced by butyrate enemas, characterized as a useful novel tool for studying IBS[1].

Butyrate is absorbed by colonic mucosa by an antiport transport against H+ inducing a pH decrease in colon observed both in the rat model and in IBS patients. This acidification of the colon could be responsible of colonic hypersensitivity. Consequently, the involvement of acid sensible ionic channels (ASICs) was investigated in the model of colonic hypersensitivity.

Methods: Rats received enemas of a 200 mM butyrate solution twice daily for 3 days and colonic hypersensitivity was evaluated at the end of intracolonic treatment by a colorectal distension test. Firstly, pCTX1a (40 µg and 80 µg, intrathecal route, a blocker of ASIC1A channel was administered 15 min before the colorectal distension test. Secondly, we investigated the role of NGF in this hypersensitivity by blocking its expression with a specific antibody in an acute (30 min before DCR test) or chronic (during butyrate treatment) manner at a dose of 1/2000° by intraperitoneal route. Dorsal spinal cord and lumbo-sacral dorsal root ganglia (DRG) of hypersensitive rats were dissected in order to analyse expression of ASICs channels by RT-PCR and hybridization *in situ* (HIS) was performed on DRG in order to localize their nociceptive fiber expression.

Results: Butyrate enemas induced a colonic hypersensitivity with a colonic reaction threshold of 43.7 ± 2 mmHg at the end of intracolonic instillations. Control rats treated with saline by intracolonic route during 3 days had a colonic reaction threshold around 60 mmHg. pCTX1a at the dose of 40 µg and 80 µg significantly reversed the decrease of colonic distension threshold in butyrate-treated rats (59.5 ± 5.6 and 62.7 ± 3 respectively vs. vehicle score, $P < 0.05$). Secondly, acute and chronic treatment with NGF-antibody significantly reversed the decrease of colonic distension threshold in butyrate-treated rats. HIS shows that the ASICs 1A and 1B is mainly expressed in nociceptives fibers of small diameters, involved in the colonic hypersensitivity induced by butyrate[1]. The RT-PCR analysis reveals a surexpression of the ASIC1A and 2B subunits in the dorsal spinal cord.

Conclusion: ASICs and NGF were involved in the development of colonic hypersensitivity induced by butyrate in the Rat.

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Strontium ranelate promotes human osteoblast replication and decreases their osteoclastogenic abilities in primary human osteoblasts

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Introduction: Strontium ranelate reduces vertebral and hip fractures in post-menopausal women by simultaneously increasing bone formation and decreasing bone resorption. To elucidate the mechanism of action involved at the cellular level in this dual activity, the current study assessed the strontium ranelate effects on replication, osteoprotegerin mRNA expression, and receptor activator of nuclear factor-kappa B ligand mRNA expression in primary human osteoblasts.

Methods: Human osteoblasts were cultured in Dulbecco's modified Eagles medium with 10% foetal bovine serum, adapted to serum-free and calcium-free medium for 24 hours, and finally, treated with strontium ranelate in physiological Ca^{2+} (1 mM). Cell replication was assessed by tritiated thymidine incorporation. The expression of osteoprotegerin and receptor activator of nuclear factor-kappa B ligand were measured using real time reverse transcription polymerase chain reaction.

Results: After a 48 hours treatment, strontium ranelate increased human osteoblasts replication in a dose-dependent manner, up to 3.8-fold with 2 mM Sr^{2+} ($P < 0.05$ vs. vehicle). Concomitantly, strontium ranelate dose-dependently increased osteoprotegerin mRNA expression, up to 1.6-fold with 1 mM Sr^{2+} ($P < 0.001$), after a 24-hour-treatment. Under the same conditions, calcium chloride (1 mM Ca^{2+}) had a lower effect. After a 24-hour-treatment with strontium ranelate (1 mM Sr^{2+}), receptor activator of nuclear factor-kappa B ligand expression was dramatically decreased compared with receptor activator of nuclear factor-kappa B ligand expression observed in vehicle (with strontium ranelate concentrations ≥0.1 mM, remaining receptor activator of nuclear factor-kappa B ligand expression was <10% of the vehicle), whereas calcium chloride (1 mM Ca^{2+}) had a less marked effect on this parameter. Overall, these results strongly support that strontium ranelate: (i) promotes primary human osteoblast replication; (ii) both increases osteoprotegerin expression and decreases receptor activator of nuclear factor-kappa B ligand expression; and (iii) is more potent than calcium in increasing osteoprotegerin expression and decreasing receptor activator of nuclear factor-kappa B ligand expression.

Conclusion: These findings show that strontium ranelate increases human osteoblasts replication while decreasing their ability to promote osteoclastogenesis. This mechanism could represent a clue for the dual mechanism of action of strontium ranelate.

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Characterization of a relevant animal model of type 2 diabetes for evaluating novel pharmacological interventions

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Introduction: An animal model mimicking the evolution of human type 2 diabetes is of great interest to test novel antidiabetic compounds. One of the classical models often used is the male obese Zucker diabetic fatty (ZDF) rat. However, the alteration of metabolic parameters (hyper insulinemia and hyperglycaemia) rapidly evolves to a drastic insulin deficiency, whereas a more stable state is needed to properly evaluate a new therapeutic agent. The present study aimed at evaluating the diabetogenic effects of a moderately high-fat diet in ZDF female rats, which are normoglycaemic in the basal state, and to validate this model using metformin as a reference treatment.

Methods: The metabolic parameters of female ZDF rats under fat diet (27.6 kcal % fat) for 8 weeks were compared with those of control animals fed with normal diet ($n = 6$ in each group).

Results: The results show an increase in weight gain (+11%, $P < 0.05$), an increase in hyperglycaemia (12.3 ± 1.3 vs. 9.0 ± 0.9 mmol/L, $P < 0.01$), a clear hyperinsulinemia (7.5 ± 1.3 ng/mL) and elevated triglyceridemia (27.2 ± 3.4 vs. 5.2 ± 0.5 mmol/L, $P < 0.01$). Glucose tolerance test (1 g/kg i.p.) revealed glucose intolerance in high-fat fed animals. After 6 weeks of high-fat diet, an oral treatment with metformin (200 mg/kg/day for 2 weeks) induced a significant decrease in hyperglycaemia ($P < 0.05$), in hyperinsulinemia ($P < 0.01$) and clearly improved glucose tolerance ($P < 0.01$). Metformin also stabilized the evolution of hyper-triglyceridemia.

Conclusion: In conclusion, ZDF female rats fed with this moderately high fat diet may be a model of interest for the *in vivo* testing of novel antidiabetic compounds.

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Effect of Ceiba pentandra extract on streptozotocin-induced type-1 diabetes in rats

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Introduction: In underdeveloped countries, more than 80% still use medicinal plants for their primary health care. *Ceiba pendandra* is a medicinal plant, empirically used to treat diabetes mellitus in Cameroon. The present study was undertaken to evaluate the effect of methylene chloride/methanol extract of *C. pendandra* extract on type 1 diabetic rats.

Methods: Diabetes was induced by intravenous streptozotocin (55 mg/kg) in adult male albino Wistar rats. Diabetic rats were divided in four groups of five animals each and treated for 35 days as follow: vehicle (diabetic control), plant extract at the doses of 40 and 75 mg/kg and insulin (10 IU/kg sc). A group of normal rats was also used as positive control.

Results: Daily administration of methylene chloride/methanol extract of *C. pentandra* significantly reduced the food and water intakes and the volume of urine excreted as well as the levels of blood glucose (71% reduction) and serum lipid profile (37% reduction), in comparison with diabetic controls. Plant extract treatment increased the level of hepatic glycogen. Immunohistochemistry of pancreas reveals a regeneration/revitalization of ß-cells.

Conclusion: The study concluded that *C. pentandra* possesses antidiabetic activity and could be a potential source to develop new oral antidiabetic agent in the treatment of diabetes mellitus.

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Involvement of indoleamine-2.3-dioxygenase activation in the CD200 Receptor cell surface expression in primary culture of human macrophages: impact in immuno-tolerance?

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Introduction: The immunosuppressive pathway mediated by the kynurenine pathway (KP) plays a major role in the immunobiology of tolerance. The first and rate limiting step in this pathway involves indoleamine-2,3-dioxygenase (IDO). IDO catabolize the oxidative cleavage of tryptophan indole ring into kynurenine. This enzyme is expressed in macrophages, and may serve as a negative regulator of the immune system. IDO has been shown to contribute to successful allopregnancy and is suspected to be involved in T cell-resistant tumours. CD200/CD200R, which helps to control myeloid function, is described as an activator of IDO. Human macrophages were used to investigate the effects of IDO activation, mediated by chronic IFN-γ treatment, on CD200R cell-surface expression.

Methods: Human peripheral blood mononuclear cells were isolated from healthy seronegative donors by ficoll-hypaque density gradient centrifugation. Monocytes were separated from PBMC by successive adherences and macrophages were obtained after 7 days of differentiation. These macrophages were cultured in a serum-free medium and were treated during 7 days with human recombinant IFN-γ (100 UI/mL). IDO activity was evaluated using HPLC measurement of tryptophan and kynurenine. IDO mRNA were quantified with RT-PCR. CD200R cell surface expression and intracellular IDO was evaluated using flow cytometer.

Results: In untreated macrophages, IDO mRNA were ubiquitously produced at low levels, IDO protein and enzyme activity were no detectable. By contrast with IDO expression, CD200R was detected. A massive IDO activation associated with *de novo* protein synthesis were observed during chronic IFN-γ treatment. The constitutive CD200R expression was down-regulated during this IDO activation.

Conclusion: These results suggest that human macrophages do not simultaneously express CD200R and IDO. The inverse expression patterns between CD200R and IDO in two distinct macrophage populations may play an important role in regulating the acquired immune system and in the control of successful allopregnancy or tumor growth.

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SR59119A, an ADRB3 agonist, reverses myometrial TNF-alpha-dependent apoptosis, and cytokine over-expression in a LPS human *in vitro* model of inflammation/infection

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Introduction: Preterm delivery remains a major cause of neonatal mortality and morbidity. Infection is one of the leading causes of preterm labour. Recent data have suggested inflammation/infection-induced apoptosis to play a role in triggering labour. This study was aimed to characterize, *In vitro*, lipopolysaccharide (LPS) - induced apoptosis in human near-term myometrium and to assess the ability of ADRB3 agonists to interfere with these processes.

Methods: Myometrium biopsies were obtained from pregnant women who delivered by caesarean section. Biopsies were placed in a 24 well plate and leaved to stabilize at 37°C for 48 h, then stimulated with LPS 10 μg/mL in the presence, or not, of SAR59119A (10^{-7} to 10^{-5} M) or TNF-alpha antibody (TNF-alpha-ab, 0.6 ng/mL) in order to characterise TNF-alpha involvement in the observed effects. Apoptosis was assessed both by real time PCR for quantitative mRNA expression and protein level expression of cleaved caspase-3. Cytokines production was assessed in supernatant using flow-cytometry.
Results: Compared with controls, LPS stimulation was associated with a significant increase of cleaved caspase-3 protein (in ADU 941.6 ± 134 vs. 452 ± 50.6 for LPS and control group respectively, $P < 0.05$) and mRNA (1.55 ± 0.32-fold increase vs. Control) expression. LPS-induced cleaved caspase-3 over-expression was abolished by TNF-alpha-ab (in ADU, 533 ± 46.9, 926 ± 126.5 and 434 ± 49.7 for control, LPS and LPS+ TNF-alpha-ab groups respectively, $P < 0.05$) and strongly reduced, in a concentration dependent manner, by SR59119A, an ADRB3 agonist (in ADU 941.6 ± 134, 824.6 ± 131.8, 694.4 ± 57.6, 525.8 ± 109 for LPS and LPS + SR59119A 10–7, 10–6 and 10–5 M, $P < 0.05$ for LPS vs. LPS + SR59119A 10^{-5} M). This was associated with a reduced mRNA expression: (in fold increase vs. controls) 1.55 ± 0.32, 1.72 ± 0.46, 1.13 ± 0.25 and 1.38 ± 0.34, for LPS alone and LPS + SR59119A 10^{-7}, 10^{-6} and 10^{-5} M respectively.
In our conditions LPS was associated with a significant increase of IL-4, IL-6 and IL-8 at 48 h. SR59119A strongly reduced the over-expression of IL-6 and IL-8 (in pg/mg, IL-8: 16482 vs. 5250; Il-6: 61861 vs. 33701 for LPS and LPS + SR59119A 10^{-5} M respectively).
Conclusion: This study suggests that inflammation triggers, through a TNF-alpha-dependent pathway, myometrium apoptosis that is partially reversed by the ADRB3 agonist SAR59119A. LPS stimulation is associated with an increase of some pro-inflammatory cytokines that is reversed by SR59119A. These results highlight the anti-inflammatory and antiapoptotic effects of ADRB3 agonists, suggesting a promising approach in the management of preterm labour.

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Saponin effects on apoptosis in human rheumatoid arthritis synoviocytes. Relationship with cyclooxygenase-2

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Introduction: Alterations in the apoptosis of synovial cells have been described in residential synoviocytes as well as inflammatory cells and associated with the pathogenesis of rheumatid arthritis (RA). These changes constitute hallmarks of synovial cell activation and contribute to both chronic inflammation and hyperplasia. Human rheumatoid synovial fibroblasts (RSF) are affected most prominently, and their resistance to apoptosis has been linked closely to the progressive destruction of articular cartilage. Moreover, the role of cyclooxygenase-2 (COX-2) and prostaglandins in synoviocyte death is still under investigation. This work investigated the effect of hecogenin and tigogenin (plant steroids) on the proliferation rate, apoptosis and COX-2 expression and activity.
Methods: Human RSF were isolated from fresh synovial biopsies obtained from patients undergoing hip synovectomy. Adherent RSF were obtained by enzymatic digestion of synovium. Between passages 4 and 8, after 48 h of culture, RSF were cultured with 20–100 μM hecogenin or tigogenin for 6, 12 and 24 h. A morphologic analysis was performed with phase-contrast microscopy. Apoptosis was evaluated by analysis of activation of caspase-3, 8 and 9 (R&D Systems) and DNA fragmentation (Roche Diagnostics). Expression of COX-2 was performed by Western blotting and culture supernatants were assayed for prostaglandin E_2 (PGE_2) production by EIA (Cayman).
Results: Direct observation with phase-contrast microscopy showed that cell shrinkage, cytoplasm condensation and formation of cytoplasmic filaments appeared after 40 μM hecogenin or tigogenin treatment. This phenomenon was correlated with inhibition of RSF proliferation. We observed a significative increase of initiator caspase-8 and 9 activities before a significative increase of executor caspase-3 activity. DNA fragmentation was enhanced in treated cells overtime compared to each control. Moreover, hecogenin and tigogenin increased COX-2 expression and activity. Indeed, the synthesis of PGE_2 was time-dependent and this production was significantly increased overtime after 40 μM hecogenin or tigogenin treatment.
Conclusion: Hecogenin and tigogenin, a plant steroids, induced an inhibition of human RSF cell growth with apoptosis induction. The effect of hecogenin or tigogenin was associated with caspase activation and DNA fragmentation. Moreover, we showed that saponin-induced apoptosis was COX-2 dependent.
Disclosure: this work was supported by La Société Française de Rhumatologie.

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Adrenomedullin inhibits adipogenesis under transcriptional control of insulin

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Introduction: Obesity prevalence is rapidly increasing in industrialized countries and is a significant risk factor for many serious illnesses such as diabetes and other disorders having insulin resistance as a common pathogenic denominator. Obesity is related to energy balance dysregulation, leading to increased fat mass due to hyperplasia and hypertrophy of white adipose cells. Therefore, particular attention is paid to adipokines regulating the developmental program of adipose tissue. We have previously shown that adrenomedullin (AM), a vasoactive peptide, is an adipokine. Increased fat mass and type 2 diabetes are linked to a rise in circulating AM levels. Nevertheless, the role of insulin in the regulation of adipocyte-produced AM and its consequences on white adipose tissue development have never been studied.
Methods: AM regulation of adipose cell differentiation was assessed using AM treatment of differentiating murine 3T3-F442A cell line and human adipose tissue stromal cells. This putative role of AM was further investigated by generating 3T3-F442A clones overexpressing or knocked-down for AM. In addition, we investigated AM gene expression regulation by insulin both *in vivo* and *in vitro*. Using a luciferase reporter gene assay in 3T3-F442A cells, we investigated 20 kb of the 5'-regulatory sequence of the AM promoter for the putative transcriptional regulation through interaction with insulin response elements (IREs).
Results: AM treatment of differentiating preadipocytes resulted in a decrease of adipose cells differentiation markers and clusters of differentiation. Moreover, the reduction of AM synthesis strongly accelerated adipose differentiation. These results were bolstered when overexpression of active AM peptide led to delayed differentiation. We also observed that insulin had an inhibitory effect on AM expression in isolated human adipocyte cells. This response was dose dependent and was reversed by resistin, a new anti-insulin agent. We quantified circulating AM in 'healthy' obese patients and observed a three-fold increase of AM compared to lean. Furthermore, AM plasma levels are negatively correlated to plasma insulin levels in these obese patients. The insulin-inhibitory response was also observed *in vivo* in Sprague Dawley rats but not in the insulin-resistant Zucker rat suggesting that AM expression is up-regulated in insulin-resistant adipose cells. Using AM promoter-luciferase reporter gene constructs, we have shown that the AM response to insulin is mediated by IREs.
Conclusion: We propose that AM is an anti-adipogenic factor regulated by insulin. These findings provide new insight into fat mass development and the relationship between obesity and elevated circulating AM levels in diabetic patients.

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Strontium ranelate stimulates murine osteoblast differentiation while decreasing their osteoclastogenic abilities

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Introduction: Strontium ranelate, a new treatment of postmenopausal osteoporosis, has been shown to present a dual mechanism of action increasing bone formation and decreasing bone resorption. The present study assessed strontium ranelate effects on osteoblast differentiation and their abilities to induce osteoclast differentiation.
Methods: Primary mouse calvaria cells were treated with strontium ranelate (0.1, 0.3 and 1 mM of Sr^{2+}) during either proliferation (day 1–5) or differentiation periods (day 5–21) or from early proliferation stages up to mineralized nodule formation (day 1–21). In all cases, evaluations were performed at the end of the culture period (21 days). Using reverse transcriptase polymerase chain reaction, osteoblast differentiation was quantified by evaluation of the mRNA expression level of osteoblastic markers, alkaline phosphatase, bone sialoprotein, osteocalcin and, osteoblast abilities to induce osteoclast differentiation by the mRNA expression level of receptor activator of nuclear factor-kappaB ligand. Osteoblast differentiation was also quantified by counting bone nodules.
Results: Whatever the strontium ranelate treatment period during the culture, osteoblasts expressed in a dose-dependant manner higher mRNA level of early differentiation markers (alkaline phosphatase, bone sialoprotein) (1.5–2 fold at 1 mM, $P < 0.01$ vs. control) and expressed also higher mRNA level of the late osteoblastic differentiation marker osteocalcin (1.7–3.3 fold at 1 mM $P < 0.01$, vs. control). Concomitantly, strontium ranelate stimulated bone nodules formation in a dose-dependant manner and this effect was maximum with a treatment during the entire culture period (until 3.6 fold at 1 mM, $P < 0.001$ vs. control). This shows that strontium ranelate stimulates murine osteoblastic differentiation. Moreover after strontium ranelate treatment during the entire culture period, osteoblasts expressed lower mRNA level of receptor activator of nuclear factor-kappaB ligand, a cytokine involved in osteoclast differentiation (-33% at 1 mM, $P < 0.05$ vs. control), suggesting that after strontium ranelate treatment osteoblasts had a reduced capacity to induce osteoclast differentiation.
Conclusion: These findings show that strontium ranelate has an anabolic effect on bone formation though osteoblastic differentiation together with a decrease in osteoblast abilities to induce osteoclast differentiation. This supports the dual mechanism of action of strontium ranelate on bone formation and resorption.

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Strontium ranelate effects on osteoblastic differentiation: involvement of prostaglandins

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Introduction: Strontium ranelate is a new treatment for osteoporosis that has both antiresorptive and anabolic effects. It has been previously shown that strontium ranelate increases alkaline phosphatase activity, mineralization, and prostaglandin E_2 production in marrow stromal cells.
Methods: To investigate the hypothesis that strontium ranelate exerts some of its anabolic effects on osteoblasts via prostaglandin production, we examined the ability of strontium ranelate to stimulate osteoblastic differentiation in marrow stromal cells from mice knockout for cyclooxygenase-2, the enzyme responsible of the prostaglandin E_2 production. Marrow stromal cells from 7–8 week old wild type and knockout mice were treated with or without strontium ranelate for 21 days. Measurements included alkaline phosphatase and osteocalcin mRNA expression (determined by real time polymerase chain reaction) and alizarin red staining for mineralization.
Results: After 14 days of culture, 1 and 3 mM strontium ranelate increased alkaline phosphatase mRNA expression by 2.0-fold ($P < 0.05$ vs. control) and 5.4-fold ($P < 0.01$ vs. control), respectively, in wild type cultures. In marrow stromal cells from cyclooxygenase-2 knockout mice, alkaline phosphatase mRNA expression was decreased by 50% ($P < 0.05$) compared with marrow stromal cells from wild type mice, and there was no increase in alkaline phosphatase mRNA expression with either concentration of strontium ranelate. After 21 days of culture, 3 mM strontium ranelate increased osteocalcin mRNA levels in marrow stromal cells from wild type mice 3-fold relative to controls ($P < 0.01$). The strontium ranelate-induced increase in osteocalcin expression was abrogated in

marrow stromal cells from knockout mice. Similar results for alkaline phosphatase and osteocalcin were seen when marrow stromal cells were treated for only the first 7 days of culture with strontium ranelate. Osteoblast mineralization was also increased by 1 and 3 mM strontium ranelate in wild type cultures. Mineralization was decreased in cyclooxygenase-2 knockout cultures compared to wild type cultures and the stimulation of mineralization induced by strontium ranelate was decreased in the knockout cultures.
Conclusion: To summarize, strontium ranelate increased differentiation and mineralization of marrow stromal cells from cyclooxygenase-2 wild type mice and these effects were decreased in marrow stromal cells from cyclooxygenase-2 knockout mice. Coupled with our previous data showing that strontium ranelate increases prostaglandin E_2 production, these results indicate that several strontium ranelate effects on bone formation may involve the local production and action of prostaglandins on osteoblasts.

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Effects of formoterol on repeated cadmium inhalation-induced lung inflammation and emphysema in rats
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Introduction: The aim of this study was to investigate the preventive effects of formoterol, a long-acting β_2-adrenoceptor agonist, against cadmium-induced lung inflammation and emphysema associated with MMP-2 and MMP-9 activation in rats.
Methods: Animals were randomly assigned to a control group ($n = 6$) and a Cd group ($n = 6$) exposed to nebulized vehicle (0.10% DMSO in saline) or to a 0.1% $CdCl_2$ solution, 3 times a week during 5 weeks respectively. The effects of 2 doses of formoterol inhaled before Cd ($n = 6$/group ; 1 mg/30 ml or 4 mg/30 ml of solution) on specific airway resistance (sRaw), cell counts, cytokines (IL-1, TNF-α and GM-CSF) and total protein content in bronchoalveolar lavage fluid (BALF) together with lung histomorphometry were investigated. MMP-2 and MMP-9 activities were determined by gelatine zymography.
Results: Compared with the control group, cadmium induced a significant 50% increase in sRaw with concomitant increases in counts of neutrophils ($10.7 \pm 3.9 \times 10^4$ vs $0.1 \pm 0.1 \times 10^4$ cells/ml; $P < 0.01$), macrophages ($9.0 \pm 2.4 \times 10^4$ vs $1.6 \pm 1.1 \times 10^4$ cells/ml; $P < 0.01$), total proteins (290.9 ± 24.5 vs 158.0 ± 68.7µg/ml; $P < 0.05$), MMP-2 (0.6 ± 0.2 vs 0.1 ± 0.1; $P < 0.001$) and MMP-9 (0.3 ± 0.1 vs 0.01 ± 0.01; $P < 0.001$) as well as parenchyma inflammatory cell infiltration and airspaces enlargement measured through the mean linear intercept enhancement (78.1 ± 2.3 vs 65.3 ± 6.7 µm; $P < 0.05$). No significant changes in cytokines in BALF were detected. Formoterol dose dependently abolished the effect of cadmium on sRaw and partially reduced the total cell number and neutrophils counts ($13.8 \pm 4.4 \times 10^4$vs $21 \pm 4.3 \times 10^4$ cells/ml; $P < 0.001$; $4.7 \pm 2.6 \times 10^4$ vs $10.7 \pm 3.9 \times 10^4$ cells/ml; $P < 0.05$) as well as MMP-9 activity (0.2 ± 0.1 vs 0.3 ± 0.1; $P < 0.001$).
Conclusion: In conclusion, formoterol partially reduces lung neutrophils infiltration and totally abolished airway obstruction in rats repeatedly exposed to Cd but don't prevent lung airspace enlargement, alveolar influx of macrophages and the increase in BALF proteins content. The preventive anti-inflammatory effects of formoterol could be partially mediated by an inhibitory action on MMP-9 activity.
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Inhibition of TNFalpha production induces decrease of amyloid peptide and nuclear phosphorylated PKR expressions
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Introduction: Alzheimer's disease is a complex neurodegenerative disorder pathologically identified by the presence of extracellular senile plaques composed of aggregates of the amyloid peptide and intracellular aggregates of protein tau as neurofibrillary tangles. Neuronal degeneration usually involves concomitant changes in other cells, such as inflammatory response of astrocytes and microglial cells, which induce release of inflammatory cytokines like TNFα for example. Moreover, it has been shown that extracellular amyloid peptide induced a phosphorylation and activation of double-stranded RNA-dependent protein kinase (PKR), which is then translocated in nucleus and plays a significant role in neuronal death.
Objective of our study was to investigate the role of an inhibition of the TNFα production on amyloid peptide and nuclear phosphorylated PKR expressions.
Methods: For this, SH-SY5Y APP695 cells, which overexpress the amyloid peptide, were treated by two molecules, described as inhibiting TNFα production, and were studied concerning their amyloid peptide expression by immunofluorescence and their nuclear phosphorylated PKR expression by immunoblotting.
Results: SH-SY5Y APP695 significantly express more nuclear phosphorylated PKR than mock control cells. After cells treatment by imipramine 20 and 40 µM for 2 hours and 10 and 20 µM for 24 hours, and by yohimbine 100 and 500 µM for 2 hours, we observed a decrease of the amyloid peptide expression by these cells.
Conclusion: This decrease of the amyloid peptide expression seems to be correlated with a decrease of nuclear phosphorylated PKR expression and it would be confirmed by complementary studies in neuron-microglial cocultures derived from Alzheimer transgenic mice and then *in vivo.*

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Possible involvement of lysophosphatidic acid in unilateral ureteral obstruction-induced renal fibrosis
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Introduction: Lysophosphatidic acid (LPA) is a bioactive phospholipid known to influence a number of cellular responses including proliferation, differentiation, and motility, via the activation of G-protein coupled receptors (LPA1/2/3/4). LPA is also suspected to be involved in pathologies such as atherosclerosis, obesity, cancer or renal disease (Gardell SE et al 2006).
Objectives: The aim of the present study was to analyse the possible involvement of LPA in the development of renal tubulointerstitial fibrosis.
Experimental approach: The production of LPA and the expression of LPA receptors were measured in kidneys from mice which underwent an unilateral ureteral obstruction (UUO), a widely used surgical model reproducing most of the alterations occurring during obstructive nephropathy (Bascands JL et al 2005).
Results: Eight days after surgery, a significant increase in tubulo-interstitial fibrosis markers (alpha-smooth muscle actin, type III collagen) was observed in the kidneys subjected to UUO when compared to the controlateral kidneys (mRNA. This was accompanied by a significant increase (5-fold) in LPA1 receptor- and no change in LPA2 receptor- mRNA levels measured by real time qPCR. LPA3 and LPA4 receptors were not detected in the normal and pathological kidney. In addition, the amount of LPA released- quantified by radioenzymatic assay- in the extracellular medium of kidney explants maintained in primary culture was increased 3-fold with UUO when compared to explants of controlateral kidneys. This increase in LPA release was accompanied by a parallel increase in the release of a phenanthrolin-sensitive lysophospholipase D activity, previously demonstrated for its involvement in LPA synthesis.
Conclusion: These results suggest the possible involvement of LPA and LPA1 receptors in UUO-induced tubulointerstitial fibrosis. Transgenic and pharmacological experiments are currently under process to test this hypothesis.

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Role of oxidative stress on stress-sensitive pathway activation and beta cell function in the absence of gluco- or lipo-toxicity
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Introduction: Pancreatic beta cell dysfunction and insulin resistance are the hallmark of type 2 diabetes. Under diabetic conditions, an increased flux of glucose and free acid fatty (respectively named as gluco- and lipo-toxicity) is associated with an increased production of reactive oxygen species (ROS). Oxidative stress produced by ROS triggers the activation of a number of cellular stress-sensitive pathways (c-Jun N-terminal kinases/stress activated protein kinases JNK/SAPK, p38 MAPK and nuclear factor-κB), leading to both insulin resistance and impaired insulin secretion.
Some evidence suggests that oxidative stress may precede the occurrence of diabetes, suggesting the potential role of ROS production as an inducer and not only as a consequence of diabetes. Thus, in this work, we examined the possible involvement of oxidative stress in the development of beta cell dysfunction in the absence of gluco- and lipo-toxicity.
Methods: INS-1 insulin secreting cells were incubated in the presence of various concentrations of H_2O_2 and activation (phosphorylation) of p38 MAPK and JNK was tested by western blot. Functionality and viability of cells were respectively evaluated by measuring insulin secretion and insoluble formazan crystals formation (MTT test). The effects of an antioxidant (N-acetyl cystein, NAC) and of two specific inhibitors of p38MAPK (SB203580) and JNK (SP600125) were also determined on insulin secretion.
Results: p38MAPK and JNK phosphorylation were detected as soon as 5 min after initiating incubation of cells in the presence of H_2O_2. Concentration-response curve obtained was biphasic, with a maximum level of phosphorylation occurring for 10 mM H_2O_2. Total protein expression did not change.
Insulin secretion induced by 8.3 mM glucose was inhibited when concentrations of H_2O_2 increased. Inhibition was detectable for 0.01 mM and maximal for 0.1 mM H_2O_2. Concentrations higher than 0.1 mM impaired cell viability. NAC, SB203580 and SP600125 partially prevent the impairment of cell functionality in the presence of 0.05 mM H_2O_2.
Conclusion: The present work allowed us to define experimental conditions mimicking the effect of oxidative stress on insulin secreting beta cells in the absence of gluco- and lipo-toxicity. Our data obtained in those conditions suggest a relationship between stress-sensitive pathway activation and impairment of insulin secretion.

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Renal effects of meloxicam, a COX-2 preferential inhibitor, in comparison with a non selective COX inhibitor, ketoprofen, in anesthetized piglets
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Introduction: COX-2 preferential or selective inhibitor NSAIDs have become popular because of their sparing effect on the cytoprotective prostaglandins in the gastrointestinal system. Renal effect of COX-2 selective NSAIDs have been largely covered, but conflicting data exist on renal effect of meloxicam, a COX-2 preferential inhibitor. The aim of this study was to assess the influence of intravenous meloxicam on renal parameters in an anesthetized piglet model, in comparison to a non-selective COX inhibitor, ketoprofen.
Methods: In this randomized, blinded study, 16 piglets (mean weight: 28 ± 3 kg, age: 82 ± 8 days) were divided in three groups: $n = 6$ received ketoprofen (GrK), $n = 5$ meloxicam (GrM) and $n = 5$ formed the control group (GrC) with an injection of placebo (NaCl 0.9%). The experiments were terminal and received the agreement of our Institutional Ethics Committee.
Under a balanced general anesthesia protocol and mechanical ventilation, the animals were equipped for cardiovascular, respiratory and renal functions evaluation, including: systemic (ABP) and pulmonary (PAP) arterial blood pressure, cardiac output (CO), heart rate (HR), urinary output (UO), glomerular filtration rate (GFR) by measuring the inulin, clearance and renal blood flow (RBF) respectively with para-amino-hippuric acid clearance and ultrasonic probe methods. After stabilization, the measurements were continuously monitored and compared to an initial control period lasting 20 minutes (period C). At the end of this period, ketoprofen ($2\ mg \cdot kg^{-1}$), meloxicam ($0.2\ mg \cdot kg^{-1}$) or placebo was injected intravenously. Parameters were recorded thereafter during 120 minutes divided into 6 periods of 20 minutes each (U1, U2, U3, U4, U5, U6).

Non-parametric statistical analysis included within time (Friedman) and between groups (Kruskal-Wallis) comparisons, and post-hoc contrast using Mann–Whitney tests.
Results: Cardiovascular and respiratory parameters were constant during the whole experiment. RBF decreased significantly in GrK from U1 to U6 period: the maximal decrease was on U2 period (15%: from 5.3 ± 0.3 to 4.5 ± 0.3 mL.kg-1.min^{-1}). For GrM, a temporary and significant diminution of 8% was observed for the period U1 and U2 (from 5.2 ± 0.3 to 4.7 ± 0.68 mL.kg^{-1}.min^{-1}) before coming back to baseline level. UO and GFR tended to follow the same evolution as RBF.
Conclusion: This study confirmed, even with a lightly hypovolemic anesthetized experimental model, that non selective COX inhibition is associated with larger renal side effect than COX-2 preferential inhibition. Nevertheless, a slight renal effect was noticed with meloxicam, which could be worse in hypovolemic condition. Further studies are needed in conditions of salt and water depletion in order to evaluate the impact of the preferential COX-2 inhibitor on renal parameters in critical conditions.

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Reversible down-regulation of MMP-9 and MMP-12 gene expression in human primary macrophages treated by arsenic trioxide

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Introduction: Chronic exposure to inorganic arsenic, a carcinogenic environmental contaminant present in cigarette smoke, is associated with immunosuppression. We recently demonstrate that, *in vitro*, non cytotoxic concentrations of arsenic trioxide (As2O3) markedly impairs endocytosis and phagocytosis activities of human primary macrophages, some major immune cells. In addition, As2O3-treated macrophages exhibit decreased expression of the CD71 macrophagic marker, increased expression of the monocytic CD14 marker and a typical monocytic rounded morphology, suggesting that arsenic could 'de-differentiate' human macrophages.
The aim of this study was to determine whether inorganic arsenic can alter expression of MMP-9 and MMP-12 genes, two typical markers of functional macrophages.
Methods: Human primary macrophages were obtained by differentiation of peripheral blood mononuclear cells with 800 UI/ml GM-CSF, which strongly increased MMP-9 and MPP-12 gene expressions after a 6-day culture. Macrophages were then treated with non cytotoxic concentrations of As2O3 (0.1–1 μM) for different times (8–72 h).
Results: Using real-time quantitative polymerase chain reaction, we demonstrate that arsenic potently decreases mRNA levels of MMP-9 in a time and concentration-dependent manner. Maximal down-regulation of MMP-9 gene expression was measured in macrophages treated with 1 μM As2O3 for 72 h (95.5 ± 1.7%, $n = 4$). Arsenic also decreases MMP-9 protein levels in whole cell lysates and cell supernatants. Similarly, As2O3 down-regulates expression of MMP-12 gene expression (99.3%, $n = 2$). Down-regulation of both MMP-9 and MMP-12 gene was almost fully reversed when As2O3-treated macrophages were next cultured with GM-CSF in arsenic-free medium.
Conclusion: In conclusion, our results (1) demonstrate that low concentrations of As2O3 could down-regulate expression of MMP-9 and MMP-12 genes, two typical markers of functional macrophages and (2) strengthen the idea that inorganic arsenic could 'de-differentiate' human macrophages into monocytic-like cells.

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Valvular heart regurgitation and pergolide: a French observational study in patients with Parkinson's disease and meta-analysis

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Introduction: Pergolide is an ergot-derived dopamine receptor agonist used to treat Parkinson's disease (PD) and restless leg syndrome. Following case reports, observational studies found an association between Pergolide intake and heart valve disease (HVD). However, recent studies failed to confirm this association and prevalence and risk factors of HVD associated with pergolide remain to be clarified. To investigate this issue we conducted an observational study at the Pitié-Salpétrière hospital and a meta-analysis of similar trials.
Methods: Prospective observational study in patients with Parkinson's disease treated with pergolide for more than 3 months ($n = 96$) vs. controls ($n = 50$) recruited at the Pitié-Salpétrière hospital (Paris) between April 2005 to August 2006. Main outcome: moderate to severe regurgitation grade in at least one heart valve in a standardized echocardiography performed by an observer blind for treatment. Meta-analysis: computed search of PubMed® (1966–2006) and Cochrane library to identify all prospective exposed/non-exposed studies 1) in patients treated with pergolide vs. controls and 2) reporting echocardiogaphic data. Endpoint: moderate to severe regurgitation grade in at least one valve.
Results: A total of 133 echocardiographs were analysed, 86 in the pergolide group and 47 in the control group. Moderate to severe regurgitation grade in at least one valve was found in 15 patients in the pergolide group (17%) compared to two patients (3%) in the control group [$OR = 4.74$ 95%CI (1.02–22.1), $P = 0.03$]. Moderate to severe regurgitation was independently associated with pergolide cumulative dose in pergolide group [adjusted $OR = 1.37$ 95%CI (1.04–1.81) for 10 mg/kg increase]. Three trials together with the present study were included in the meta-analysis, representing 229 patients treated with pergolide and 188 controls. The overall OR for moderate to severe regurgitation was 4.9 [95%CI (1.92–12.6), $P < 0.001$]. Risk differences were significantly correlated with means of pergolide cumulative dose in these trials ($r = 0.96$, $P < 0.01$).
Conclusion: Our study as well as the meta-analysis confirm the association between HVD in PD patients taking pergolide. Prevalence of HVD is high and correlates with pergolide cumulative dose.

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Effect of milnacipran on extracellular levels of noradrenaline, dopamine and serotonin (5-HT) in the medial prefrontal cortex of freely moving rats, as measured by intracerebral microdialysis

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Introduction: Milnacipran is a new antidepressant which selectively and equipotently inhibits the neuronal recapture of noradrenaline (NA) and 5-HT both *in vitro* and *in vivo* (Boyer & Briley, 1998. *Drugs of Today* 34: 709–720). The present study used microdialysis to examine the dose-dependency and duration of effect of single oral administrations of milnacipran (10, 20 and 40 mg/kg) on simultaneous measures of extracellular NA, dopamine (DA) and serotonin (5-HT) levels in the medial prefrontal cortex of conscious rats.
Methods: Animals were handled and cared for in accordance with the Guide for Care and Use of Laboratory Animals (National Research Council, 1996) and the European Directive N 86/609, and the experimental protocol was carried out in compliance with local ethical committee guidelines for animal research. Male Sprague-Dawley rats [Ico: OFA-SD (IOPS.Caw), IFFA CREDO; 6–10 rats per treatment group] were surgically implanted with microdialysis probes (CMA/12, 3 mm), transferred to microdialysis cages and allowed an overnight recovery period. The following morning, three 40-min dialysate samples were collected for establishing the 'baseline' measurements of monoamine levels. Immediately thereafter, milnacipran or saline vehicle was administered by oral gavage and dialysate samples were collected continuously for the next 22 h using refrigerated microfraction collectors. Monoamine levels were quantified in the dialysate samples by HPLC coupled with electrochemical (DA and 5-HT) and fluorometric (derivatized NA) detection modes. Detection limits were around 2.2 fmol/30 μL dialysate volume.
Results: Slight but significant increases in NA, DA and 5-HT levels (50–75% over baseline) were observed during the first hour following oral administration of the saline vehicle. Milnacipran resulted in significant dose-dependent increases in the levels of all three monoamines (see Table), which were maximal (160–230% over baseline) during the first 2 h post-administration, and which remained significantly elevated for up to 8 h at the higher doses.

Treatment mg/kg p.o.Mean% baseline (±SEM) during 9 h post-treatment interval

		Noradrenaline (NA)	Dopamine (DA)	Serotonin (5-HT)
Vehicle	–	108 ± 4	133 ± 14	125 ± 12
Milnacipran	10	159 ± 11**	158 ± 24	117 ± 18
	20	166 ± 11**	185 ± 17*	184 ± 24*
	40	218 ± 23**	229 ± 47*	212 ± 26**

*,**$P < 0.05$, 0.01 vs. vehicle

Conclusion: These results are consistent with and complement other microdialysis studies showing a central dose-dependent effect of milnacipran to increase extracellular levels of NA, DA and 5-HT in the prefrontal cortex of the rat following systemic administration (Kitaichi *et al.*, 2005. *Eur. J. Pharmacol.* 516: 219–226; Bel & Artigas, 1999. *Neuropsychopharmacology* 21: 745–754; Mochizuki *et al.*, 2002. *Psychopharmacology* 162: 323–332; Koch *et al.*, 2003. *Neuropharmacology* 45: 935–944), and demonstrate for the first time a 6–8 h duration of this effect following single oral administration in this species.

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Cannabis and cutting materials

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Introduction: Since several months, it is rumoured among cannabis users that glass (crushed glass or glass wood) is used as cutting material for cannabis leaves. To confirm the presence and determine chemical composition and cristalline form of these cutting materials, different cannabis samples were analysed macroscopically, microscopically and by X-ray diffraction.
Methods: Samples were obtained through the TREND project. All samples originated from different cities but all were collected in the northern part of France. Macroscopic examination (Trinocular Olympus SZX12, numeric camera Altra 20, ANALYSES GET IT® software) permitted to isolate some objects for X-ray diffraction (Enraf Nonius CAD4 and Rigaku R-Axis Rapid diffractometers). One sample was heated at 1000°C in an oven.
Results: All samples contained objects (crystals and/or micro beads) other than vegetals, in different proportions.
One sample was studied with X-ray diffraction. The parameters of the cristalllin form were:a = b = 4916 Å; c = 5401 Å; α = β = 90°; γ = 120°, corresponding to quartz alpha. Chemical composition was SiO2.
Some micro beads showed no diffraction: they were constituted of an amorphous solid, which was SiO2. Diameters of these beads varied from 0.02 to 0.1 mm in the different samples.
One sample, containing a high proportion of micro beads, was heated at 1000°C: the residual part contained partially dissolved beads.
Conclusion: This study confirms the presence of quartz crystals and/or of glass micro beads in the samples. The reason for which these surprising cutting materials were present in these samples is unknown. According to the users, it would be

added for giving a brilliant and more attractive aspect. The potential medical consequences of inhalation of SiO2 particles are unknown, although the diameter of the observed particles seems too big to go directly in the low respiratory tract.

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Fenofibrate, a PPAR alpha agonist, exerts neurological recovery-promoting, anti-inflammatory and anti-oxidative effects in traumatic brain injury

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Introduction: We previously demonstrated that fenofibrate, a peroxisome proliferator-activated receptor α (PPARα) agonist, reduced the neurological deficit, the edema and the cerebral lesion induced by traumatic brain injury (TBI). This was associated with a decreased intercellular adhesion molecule-1 (ICAM-1) expression induced by TBI. In order to further elucidate these beneficial effects, in the present study, we investigated, in the same TBI model, fenofibrate's effects on the post-traumatic inflammation and oxidative stress.
Methods: Male Sprague Dawley rats were randomized in four groups: non-operated, sham-operated, TBI + vehicle, TBI + fenofibrate. TBI was induced by lateral fluid percussion of the temporoparietal cortex. Sham-operated rats underwent the same surgery except for percussion. Rats were given fenofibrate (50 mg/kg) or its vehicle (water containing 0.2% methylcellulose) by gavage 1 and 6 h after brain injury. A neurological assessment was done 24 h after TBI (score ranging from 0 = worst to 9 = best), then rats were killed and the brain MMP9 and COX2 expression, total glutathione (GSx), oxidized glutathione (GSSG) levels were determined. The same schedule of treatment was used to evaluate the effect of fenofibrate on immunohistochemistry of 3-nitrotyrosine (3NT), 4-hydroxynonenal (4HNE), and inducible nitric oxide synthase (iNOS) at 24 h post-injury.
Results: Fenofibrate significantly improved the neurological score (7.1 ± 0.5, $n = 10$, vs. 5.0 ± 0.5, $n = 10$ for TBI rats, $P < 0.01$). In addition, fenofibrate reduced TBI-induced proMMP9 expression and activity, COX2 expression ($P < 0.05$). TBI led to a 52% decrease in the GSx content and an increase of 400% in glutathione oxidation ratio (2GSSG/GSx) showing an important oxidative stress, which were reduced by fenofibrate ($P < 0.05$). In addition, immunohistochemistry studies showed that TBI resulted in a considerable 4HNE staining, a marker of lipid peroxidation by free radicals, and 3NT staining, a marker of protein nitration by peroxynitrite anions. These two staining are markedly reduced by fenofibrate. Last but not least, TBI induced an important staining of iNOS which is also decreased by the PPARα agonist.
Conclusion: Our results showed that fenofibrate promotes neurological recovery by exerting anti-inflammatory effect evidenced by a decrease in iNOS, COX2 and MMP9 expression. In addition, fenofibrate showed anti-oxidant effects demonstrated by reduction of four markers of oxidative stress: loss of glutathione, glutathione oxidation ratio, 3NT and 4HNE staining. Our data suggest that PPARα activation could mediate pleiotropic effects and strengthen that it could be a promising therapeutic strategy for TBI.

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Coadministration benzodiazepine and antidepressant drugs: State of the art

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Introduction: The potential for drug-drug interactions in psychiatric patients is very high as combination psychopharmacotherapy used to treat comorbid psychiatric disorders, to treat the adverse effects of a medication, to augment a medication effect or to treat concomitant medical illnesses, etc. Interactions can be pharmacodynamic or pharmacokinetic in nature.
In clinical practice the benzodiazepines (BZD) are often prescribed together with antidepressant drugs for the treatment of depression. Although there has been no convincing evidence to show that such a combination is more effective than antidepressants alone and no clear indication for the use of this combination.
Yet, anxiety frequently coexists with depression, and an anxiolytic is often added. But, it is BZD which are systematically prescribed together with antidepressants. However, BZD themselves have no antidepressive effects and we lack firm evidence for or against this combination therapy.
Methods: We have rewieved all trials that compared antidepressant-benzodiazepine treatment with antidepressant alone for adult patients with major depression were sought by electronic searches of Medline and several other databases (1970–2005), combined with hand searching, reference searching and SciSearch. First, 4472 publications were selected. Second, with predefined criteria, 1416 were used.
Results: Data were issues from 410 studies. 236 ($n = 38417$) with BZD authorized and, 174 ($n = 31404$) without. Altogether, the percentage of responders, without BZD is significantly higher (59.2%; chi2 = $P < 0.001$).
In presence of BZD, noradrenergic antidepressant drugs, the% of responders there are significantly most important compared with SRI ($\chi^2 = 74.2$; $P < 0.001$).
Without BZD the % of responders is significantly higher with antidepressant drugs compared when BZD are prescribed: SRI $\chi^2 = 259.6$, $P < 0.001$; MAOI $\chi^2 = 23.8$, $P < 0.001$, ADS mixed NAD AND 5-HT antidepressant drugs, $\chi^2 = 70.3$, $P < 0.001$, tricyclics $\chi^2 = 0.28$; NS.
Conclusion: We are focused on the pharmacodynamic interactions between anxiolytic such as BZD, and antidepressant drugs which may result in a reduction of the effects of one of the drugs. It is known that BZD decrease the serotoninergic transmission. Also, it may be suggested that combined BZD-specific serotonin reuptake inhibitors, should be avoided.

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Neuroprotective effect of simvastatin in a rodent cardiopulmonary bypass model

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Introduction: Neurocognitive impairment is frequently reported following cardiopulmonary bypass. We previously demonstrated in a rodent model that cardiopulmonary bypass induced an inflammatory activation and a cerebral endothelial dysfunction, associated with a hippocampal neuronal loss. Statins have beneficial properties on reducing inflammation and enhancing endothelial dependant relaxation in cerebral ischemia reperfusion models. The purpose of this study was to assess the effects of simvastatin pre-treatment on cardiopulmonary bypass induced cerebro-vascular injuries.
Methods: Male Sprague Dawley rats were randomly allocated to the simvastatin pre-treated group (10 mg/kg/d, 14 days) or a non-treated group. Animals underwent a 30 minutes cardiopulmonary bypass procedure or sham surgery. Animals were sacrificed at the immediate post-operative period (T0) or 24 hours after the procedure (T24). *In vitro* middle cerebral artery reactivity, systemic inflammation evaluation by measuring plasma concentrations of TNFα and immuno-histochemistry studies in the CA3 of the hippocampus, using an ICAM-1 antibody and a NEU-N antibody (to assess a neurons counting), were achieved.
Results: Cardiopulmonary bypass was responsible for impairments of the middle cerebral artery endothelial function at T0 and T24, which were prevented in the simvastatin pre-treated group. The cardiopulmonary bypass induced release of TNFα and overexpression of ICAM-1 which were both decreased in the simvastatin pre-treated groups. The T24 cardiopulmonary bypass induced neuronal loss, revealed by hippocampal neurons counting, was also prevented by simvastatin.
Conclusion: Simvastatin is efficient to alleviate cerebro-vascular injuries of cardiopulmonary bypass. This treatment could be a therapeutic option to avoid neurocognitive impairments following cardiopulmonary bypass.

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Agomelatine efficacy on major sleep disturbances in Smith-Magenis syndrome: an exploratory, open study in children

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Introduction: Smith-Magenis syndrome (SMS) is a mental retardation disease with distinctive behavioural characteristics, dysmorphic features and congenital anomalies ascribed to a deletion of chromosome 17p11.2. Severe sleep disturbances and maladaptive daytime behaviour have been linked to an abnormal circadian pattern of melatonin, with a diurnal instead of a nocturnal secretion of this hormone. An open clinical trial was designed to assess the potential efficacy of agomelatine on the sleep disturbances in children suffering from SMS.
Methods: Open phase II study with direct individual benefit without randomisation. All patients were treated during 6-month with agomelatine (1 or 5 mg o.d. in the evening). Acebutolol (10 mg/kg o.d. in the morning) (ß1 antagonist) was co-administered in order to block endogeneous melatonin secretion. The primary efficacy criteria were the actigraphy parameters.
Results: Seven male and three female SMS patients, aged from 6 to 17 years, were included. Nine patients completed the 6-month study whereas one patient withdrew from the study due to non-medical reason before having taken any dose of agomelatine. The results from actigraphy were consistent with those obtained with the sleep diary and the children's sleep questionnaire, which showed that the nocturnal waking up was less frequent and shorter than at baseline, and that the mean duration of the naps decreased over the 6-month study period. According to the investigator, clinical improvement in the children was notable and the parents of every child confirmed the benefit of the treatment. The children slept deeply and were quiet whereas in the past it used to be dramatic. The sleep was no more fragmented by prolonged nocturnal awakenings and waking-up in the morning was delayed.
Conclusion: Data from this exploratory, open trial indicate that agomelatine (1 or 5 mg), when co-administered with acebutolol (10 mg/kg), was an effective and well-tolerated treatment of sleep disturbances in SMS. On the family requests, eight patients are at present still treated and receive agomelatine since November 2002. Additional trials are needed to confirm the therapeutic potential of agomelatine in SMS.

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Addition of benzodiazepines to buprenorphine induces potentiation of lethal doses after IP administration in mice but changes in μ opiate receptor binding are not dose dependent excepted in thalamus

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Introduction: BPN is a partial μ agonist and a delta + kappa antagonist, which binds to opioid receptors (OR) with high affinity. BPN is widely used in France for opiate substitution therapy with bad consequences like numerous misuses and some overdoses. (i) We investigated changes in lethal doses of BPN after co-administration of a potent BZD i.e. flunitrazepam (FNZ) in mice. (ii) Previously we established clorazepate (CRZ) has a direct effect on μ opioid receptor (MOR) binding [e.g. In amygdaloid nucleus Kd = x2.3 and Bmax −30% and in thalamus Kd x2 with a paradoxical increase in Bmax (+60% and +80% in acute and chronic conditions respectively)]. We tested whether this effect on MOR binding was dose dependent using increasing doses of CRZ and the BZD antagonist flumazenil.
Methods: (i) In mice various non lethal dosages of FNZ (25–100 mg/kg) were associated to infra-DL_{50} BPN dosage (i.e. 20–100 mg/kg) IP; we measured the acute lethality until 8 days. (ii) CRZ administration from 2 to 60 mg/kg or flumazenil 0.3 mg to 3 mg/kg, once IP. Rats were sacrificed 6 h post injection. Using a β-imager 2000 (BIOSPACE Instruments, Paris), we measured on frozen brain sections Kd and Bmax for MOR with respect to the various functional regions implicated in emotional behaviour and memory.
Results: (i) Lethal effect of FNZ added to BPN 100 mg/kg in mice: the percentage of dead animal increased from 25 to 100% in the 50–200 mg/kg of FNZ dose range. (ii) MOR binding: in all regions we failed to find a dose dependent effect in Kd fluctuation; nevertheless in Thalamus a significant dose dependant increase in Bmax was observed with CRZ and a significant decrease with flumazenil.
Conclusion: (i) IP administration of flunitrazepam in addition to buprenorphine induces lethal dose potentiation. While this μ partial agonist is able to induce death by apnoea, the potentiation of lethal doses by the BZD is clear with 1/40–1/10 doses of its LD_{50}. As the shape of the BPN lethal dose curve is especially steeply it

seems the addition of BZD could smooth the slope but with the addition of delayed deaths. (ii) After Clorazepate IP administration the MOR binding changes observed 6 h post-injection were not significant. Previously the changes induced in Kd and Bmax were observed 24 h after IP injection. So, the changes in MOR regulation induced by BZD could require a long delay.

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Activation of nucleus tractus solitarius 5-ht$_{2a}$ but not other 5-ht2 receptor subtypes inhibits the sympathetic activity in rats

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Introduction: In previous studies, we showed that nucleus tractus solitarius (NTS) 5-HT$_3$ receptor stimulation elicits a sympatho excitation, without affecting heart rate. Conversely, the activation of NTS 5-HT$_2$ receptors produces the typical responses of baroreceptor activation: hypotension and bradycardia. However, to date, the receptor subtype underlying the cardiovascular responses to 5-HT$_2$ receptor stimulation in the NTS remained to be identified.

Methods: In order to address this question, we first analysed, in pentobarbitone-anaesthetized rats, the effects of intra-NTS microinjections of selective agonists of the different 5-HT$_2$ receptor subtypes, on BP and HR baselines.

Results: Under these conditions, 2,5-dimethoxy-4-iodoamphetamine (DOI), a wide spectrum 5-HT$_2$ receptor agonist, but not selective 5-HT$_{2B}$ and 5-HT$_{2C}$ receptor agonists, produced a decrease in blood pressure and in heart rate. The maximal cardiovascular changes evoked by DOI (0.5 pmol) could be almost completely abolished by prior intra-NTS microinjection (10 pmol) of MDL-100907, a selective 5-HT$_{2A}$ receptor antagonist, but not by 5-HT$_{2B}$ or 5-HT$_{2C}$ receptor antagonists. In addition, using extracellular recordings, we found that a large majority of identified cardiovascular rostro-ventrolateral medulla (RVLM) neurons were almost totally inhibited by NTS 5-HT$_{2A}$ receptor stimulation. We then investigated whether intra-NTS administration of a subthreshold (0.05 pmol) dose of DOI, known to facilitate the cardiovagal component of the baroreflex, could also modulate the sympathoinhibitory component of this reflex. These experiments showed that neither the decrease of the cardiovascular RVLM neuron and lumbar sympathetic nerve activities produced by i.v. Phenylephrine administration (gain of the baroreflex), nor the hypotensive response elicited by aortic nerve stimulation were potentiated by intra-NTS microinjection of DOI under such conditions.

Conclusion: These data showed that activation of 5-HT$_{2A}$, but not 5-HT$_{2B}$ or 5-HT$_{2C}$, receptors, located on NTS neurons, elicits depressor and bradycardic responses, and that the 5-HT$_{2A}$-mediated hypotension is produced via the inhibition of RVLM cardiovascular neurons. However, the negative data with the subthreshold dose of DOI indicate that sympathetic baroreflex NTS neurons are probably not endowed with 5-HT$_{2A}$ receptors.

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Agomelatine in generalized anxiety disorder: a randomized, placebo-controlled, study with a possibility for blinded dose-adjustment

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Introduction: Agomelatine is a novel agent that acts on melatonin (MT$_1$, MT$_2$) receptors and serotonin (5-HT$_{2C}$) receptors. Preclinical data and data from clinical trials in major depression suggest that agomelatine may have anxiolytic properties. This randomised, double-blind placebo-controlled clinical trial was designed to assess the agomelatine potential efficacy in generalized anxiety disorder (GAD).

Methods: A total of 121 non-depressed patients with DSM-IV GAD were randomised to agomelatine (25–50 mg/day) or placebo for 12 weeks. The primary outcome measure was the Hamilton Anxiety Rating Scale (HAM-A), while secondary outcome measures included the clinical global impression (CGI) scales, the leeds sleep evaluation questionnaires (LSEQ), and the Sheehan Disability Scales (SDS).

Results: The main analysis on the last HAM-A total score change from baseline demonstrated the superiority of agomelatine 25–50 mg as compared to placebo [E (SE) = −3.28 (1.58); 95%CI = (−6.41; −0.15), P = 0.040, ANCOVA].

Data on secondary criteria, including clinical response, symptoms of insomnia, and improvement in associated disability supported the efficacy of agomelatine. Of particular note, agomelatine was tolerated as well as placebo, and was not associated with discontinuation emergent symptoms.

Conclusion: Data from this trial indicate that agomelatine is effective and very well-tolerated in the treatment of generalized anxiety disorder. Additional trials, using an active comparator, and extending over a longer period of time, are needed in order to delineate the place of agomelatine in the contemporary pharmacotherapy of anxiety disorders.

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***Lactobacillus acidophilus* modulates intestinal pain and induces opioid and cannabinoid receptors**

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Introduction: Abdominal pain is a common symptom attributed to visceral hypersensitivity. Specific probiotic administration may prevent abdominal symptoms observed in irritable bowel syndrome, the most common gastrointestinal disease.

Methods: The ability of five well known and representative probiotic bacteria belonging to the *Lactobacillus* and *Bifidobacterium* genus, compared to commensal and adherent-invasive (LF82) *Escherichia coli (E. coli)*, to induce expression of analgesic receptors (MOR and CB2 receptors) was evaluated on human HT-29 epithelial cells. The functional role of *L. acidophilus* NCFM-induced analgesic receptors was investigated by assessing the colonic perception of rats using a validated technique of colorectal distension.

Results: *L. acidophilus* NCFM induced a sustained increase of *OPRM1* mRNA expression one hour after bacterial stimulation, this induction was of the same magnitude as that observed with the positive i.e. TNF-a. NCFMstrain was able to induce significant *CNR2* mRNA expression compared to resting epithelial cells. Expression of opioid receptor, mu1 (MOR1) and CB2 was detected on HT-29 epithelial cells incubated with the *L. acidophilus* NCFMstrain. In rodents, *L. acidophilus* NCFM administration at a clinically relevant concentration of 10^9 CFU / d during 15 constitutive days induced MOR1 and CB2 protein expression in 25–60% of epithelial cells. In untreated rats, a mean colorectal distension of 50 ± 2 mmHg was required to induce pain characterized by clear visible abdominal contraction and elevation of the hind part of the animal body. Oral administration of the *L. acidophilus* NCFM strain (10^9 CFU/d) during 15 days decreased the normal visceral perception allowing a 20% increase of this pain threshold. In a model of chronic colonic hypersensitivity elicited by butyrate enemas and mimicking irritable bowel syndrome, hypersensitivity of rats was improved by the *L. acidophilus* NCFM strain which increased by 44% the colorectal distension threshold compared to untreated animals, exerted an antinociceptive effect at the same magnitude as 1 mg/kg of morphine administered subcutaneously and enhanced by 65% the suboptimal analgesic effects of morphine used at 0.1 mg/kg. *L. acidophilus* NCFM-induced analgesia was significantly inhibited by peritoneal administration of the CB2-selective antagonist but not by the opioid receptor antagonist naloxone methiodide.

Conclusion: These results suggest that the microbiology of the intestinal tract influences our visceral perception and open new perspectives in the management of abdominal pain and irritable bowel syndrome.

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Consumption of benzodiazepines among drug addicts in Ile de France area: course over 5 years

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Introduction: The centers of evaluation and information on pharmacodependance (CEIP: national network in drug dependence monitoring) developed tools allowing the monitoring of the psycho-active substances misuse. One of them, OPPIDUM programme (observation of illegal drugs and misuse psychotropic medications) is an annual, national and multicentric pharmacoepidemiological study describing consumption profile of substances.

Methods: We propose to examine with OPPIDUM the evolution of the consumption of benzodiazepines and related (BZD) among the patients consulting in structures of care specialized in Island of France between 2000 and 2004.

Results: A total of 29% of outpatients reported to consume at least one BZD. They are mostly men (72%) and their mean age is 37.4 years. 61% of them lived under unfavourable socio-economic conditions. A polyconsommation of psycho-active substances is noted in 96% of the cases with an average number of products of 3.3. The BZD are consumed orally in 98%, in a daily way in 82% and as more than 1 year in 55% of the cases. The BZD are obtained by illicite way in 13%. Whereas the flunitrazepam was the BZD of choice for the drug addicts during the Nineties, it is noticed that its consumption decreases. Other BZD such as the bromazepam (14% in 2000, 21% in 2003) is taking over. Consumption of the clonazepam and zopiclone seems to increase gradually and needs some attention.

Conclusion: The new health regulation limiting the prescription and the delivery of the flunitrazepam lead to its decrease use but seems to be replaced by other BZD such as bromazepam. This trend is confirmed by the Nots data (other data of spontaneous notification).

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Disregulation in sympathetic activity explains gender differences in irs-2 knockout mice

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Introduction: Deletion of insulin receptor substrate *Irs2* in mice causes insulin resistance in liver and adipose tissue and a profound decrease in β-cell mass, which culminates in diabetes. However, this diabetic phenotype displays a sexual dimorphism; most male *Irs-2* knockouts (KO) are diabetic by 12 weeks of age and die by 16 weeks whereas female KOs develop a milder form of diabetes and many live up to 6 months.

Methods: In this work we investigate β-cell function and lipolysis in male and female *Irs2*$^{-/-}$ mice as potential physiological explanations for the sexual dimorphism in this diabetic model. Experiments on fat cells lypolysis and insulin secretion from isolated islet were carried out according to Iglesias-Osma et al. 2004.

Results: The reduction in β-cell mass produced by the absence of *Irs-2* signals was less severe in female than in male KOs (28% vs. 40%). Moreover, our studies revealed that insulin secretion differs between male and female KO islets. Analysis of glucose-stimulated insulin release demonstrated that male *Irs-2* KO secreted more insulin than their WT controls when challenged with various concentrations of glucose. In contrast, insulin secretion in female KO islets was equivalent to female controls. However, total insulin content was equivalently reduced in both male and female KO islets. The α?2 adrenergic agonist UK 14304 (10 μM) inhibited less efficiently glucose induced insulin secretion in male *Irs-2* KO islets than in their WT control (38% vs. 67%) whereas carbachol (10 μM) only elicited a greater insulin-secretory response in *Irs2*$^{-/-}$ males (76% vs. 53% in WT). Consistent with this, basal lipolysis was enhanced in male KOs but decreased in female KOs in comparison to their respective WT controls. Adiponectin plasma levels were also significantly higher in female KOs. The elevated basal lipolysis reflects pronounced insulin resistance in adipose tissue of pre-diabetic male KOs. Moreover, isoprenaline and forskoline evoked fat cells lipolysis from female KOs was severely attenuated. Interestingly, expression of the β3 adrenoceptor was greatly reduced in KO female adipocytes in comparison to WT females, KO males and WT males. In addition, the expression of protein kinase A was suppressed in females KO *Irs-2*.

Conclusion: Dysregulation of sympathetic activity leading to alterations in beta-cell and adipocyte functions would help to understand the gender differences above reported.

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Pharmacological screening of two novel 1, 5-benzodiazepin-2-ones. Assessment of the hypnotic and the anticonvulsant activities in mice

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Introduction: The present work aims to investigate whether two agents of novel 1, 5-benzodiazepin-2-ones: the 4- (2-hydroxyphenyl) -1, 5 benzodiazepine-2-one (called 4a) and the Benzopyrano [4, 3-c] 1, 5 benzodiazepine (called 5a) have interesting pharmacological activity in mice.

Methods: Both molecules 4a and 5a have been prepared according to simple methods of synthesis using 4-hydroxycoumarine as reagent. Each molecule is tested for both anticonvulsant and hypnotic activities. A total of 250 mice Swiss albinos (171male and 79 female), 10–12 weeks old, has been used. The hypnotic activity is assessed by the effect of 4a or 5a injection (0.1–100 mg/kg, *i.p*) on the increase of Hexobarbital-induced narcosis (20 mg/kg, s.c). For the anticonvulsant activity, a dose of 4a or 5a (6.25–75 mg/kg) is injected by *i.p* route prior treatment with the convulsant agent Pentylenetetrazole (85 mg/kg, *s.c*). The two activities were respectively compared to those of flunitrazepam and diazepam as drug references. The data are mainly analysed according to student's *t* and chi-squared tests.

Results: The increase of sleep duration induced by hexobarbital is significantly higher with the molecule 4a as compared to 5a ($P < 0.01$). The efficacy dose ED_{50} of 4a (1.7 mg/kg) is lower than that of 5a (2.63 mg/kg). However, the anticonvulsant activity of 5a is more pronounced than that of 4a in the range-dose of (20–37.5 mg/kg). From the dose 50 mg/kg, a total protection was observed against the clonic, tonic seizures and mortality ($P \leq 0.05$).

The difference between the two novel 1, 5-benzodiazepines and the drug references could be related to the lack of specific pharmacophores which generate a less pharmacological activity. The variation in 4a and 5a activities may be related to their spatial conformation since 5a has a plane conformation whereas 4a has three-dimensional one.

Conclusion: The present study showed the importance on the structure-activity relationship in the hypnotic and anticonvulsant activities of the novel 1, 5-benzodiazepin-2-one. Further pharmacological studies are required to explain the role of the pharmacophores in the mechanisms of benzodiazepines activities.

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Inhibition of cardiac reflex responses by direct stimulation of periaqueductal gray and cuneiformis nucleus in rats

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Introduction: Dorsal periaqueductal gray (dPAG) stimulation in anaesthetized rats elicits 'arousal-like' (piloerection, exophthalmia) and cardiovascular responses (increase in blood pressure and heart rate), which are characteristic active events triggered by stressful situations (i.e. defense reaction). We showed previously that dPAG stimulation produces also the inhibition of the cardiac (bradycardia) response of a reflex critically involved in cardiovascular homeostasis, the baroreflex. In addition, our studies indicated that dPAG-mediated inhibition of the baroreflex bradycardia resulted primarily from the activation of $5\text{-}HT_3$ receptors in the nucleus tractus solitarius (NTS), by serotonin released from afferences originating in the B3 region (which includes the raphe magnus and paragigantocellularis nuclei). These results suggested that dPAG stimulation could also inhibit the cardiac component of the Bezold-Jarisch reflex, another vital cardiovascular reflex, through B3 activation.

Methods: We therefore analyzed the effect of dPAG stimulation (50 Hz, 1 ms pulse duration, 70 μA) on the Bezold-Jarisch reflex bradycardia evoked by phenylbiguanide (20 μg/kg, i.v.), before and after microinjections into the NTS of $5\text{-}HT_3$-, $GABA_A$- and NK_1-receptor antagonists, and following B3 chemical blockade by muscimol (5 mM).

Results: The Bezold-Jarisch bradycardia was strongly inhibited (−80%) during dPAG electrical stimulation. Intra-NTS microinjections of granisetron (175 pmol), a $5\text{-}HT_3$ receptor antagonist, bicuculline (5 pmol), a $GABA_A$ receptor antagonist, as well as GR205171 (10 pmol), a NK_1 receptor antagonist, prevented the inhibitory effect of dPAG stimulation. Muscimol microinjection into B3 also prevented dPAG-mediated inhibition. In contrast, the cardiac reflex response was inhibited (−75%) following B3 activation by DL-Homocysteic acid (30 mM), and the effect of B3 activation could be dose-dependently prevented by intra-NTS microinjections of granisetron (175–250 pmol). Using anterograde (Phaseolus vulgaris leucoagglutinin) tracer, we found that the cuneiformis nucleus sends direct projections to dPAG. Interestingly, direct electrical stimulation of the cuneiformis nucleus also produced the same 'arousal-like' and cardiovascular [i.e. inhibition of the baroreflex (75%) and Bezold-Jarisch reflex (80%) bradycardia] responses as those occurring during the defense reaction.

Conclusion: Altogether, these results suggest that, in stressful situations, activation of the cuneiformis nucleus may produce dPAG neuroexcitation, which causes (i) B3 activation at the origin of 5-HT release within the NTS, (ii) local $5\text{-}HT_3$ receptor stimulation and (iii) consequent inhibition, via $GABA_A$ and NK_1 receptor activation, of cardiac reflex bradycardia.

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TRAAK, a potassium channel involved in polymodal pain perception

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Introduction: Ion channels play a very important role in the detection of pain. TREK-1, TREK-2 and TRAAK are members of the two-pore domain K^+ (K2P) channel family and are activated by membrane stretch and free fatty acids. TREK-1 has been shown to be sensitive to temperature in expression systems. TREK-2 and TRAAK are also temperature-sensitive channels, are active at physiological body temperature, and therefore would contribute to the background K^+ conductance and regulate cell excitability in response to various physical and chemical stimuli. The purpose of this work was to make use of TRAAK knockout mice and TREK-1/TRAAK double knock-out mice to evaluate the exact role of these K^+ channels in pain perception associated with different types of stimuli.

Methods: All experiments were performed on 20–24 g male C57B/l6 J mice, TRAAK knockout mice and TREK-1/TRAAK double knock-out mice of the N10 F2 backcross generation to C57Bl/6 J congenic strain. All mice were acclimatized to the laboratory conditions for at least 1 week prior to testing. They were housed in grouped cages in a temperature-controlled environment with food and water *ad libitum*. The behavioral experiments (thermal, mechanical and chemical pain tests associated or not with two models of inflammatory pain) were performed blind to the genotype, in a quiet room, by the same experimenter for a given test taking great care to avoid or minimize discomfort of the animals.

Results: $TRAAK^{-/-}$ mice were hypersensitive to thermal pain, displaying a faster reaction time to withdraw their tail from hot temperature baths at 46, 48 or 50°C. By touching the skin with von Frey hairs of increasing stiffness, the withdrawal threshold decreases from 0.86 to 0.40 g in $TRAAK^{-/-}$ mice. We also found that the interphase of the formalin test, a period of inactivity that follows the initial intense period of pain behavior, is greatly reduced in the TRAAK-null mutant mice. $TRAAK^{-/-}$ mice were also more sensitive to acetic acid injection in the peritoneal cavity than $TRAAK^{+/+}$ mice. Both $TRAAK^{+/+}$ and $TRAAK^{-/-}$ mice, injected intraplantarly with carrageenan to produce inflammation showed thermal hyperalgesia (46°C). The carrageenan-induced decrease in reaction score was lower in $TRAAK^{-/-}$ mice. An exacerbation of the sensibility observed in these tests and models of pain was observed in the double knock-out mice.

Conclusion: Like TREK-1, TRAAK appears as an important ion channel for polymodal pain perception and as an attractive target for the development of new analgesics.

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Levodopa effect on motor activity in Parkinsonism: a PET study

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Introduction: Pharmacological and physiopathological mechanisms underlying multiple system atrophy (MSA) are poorly understood. Even levodopa is not completely effective, the effect of levodopa on regional cerebral blood flow (rCBF) during motor task needs to be assessed. This prospective study investigated levodopa effect on motor activation in three groups of subjects: MSA, Parkinson's disease (PD) and healthy volunteers.

Methods: Eighteen MSA patients (66 ± 9 years) and nine PD patients (62 ± 8 years) were studied in two conditions OFF (without dopaminergic treatment) and ON (after levodopa). For each condition, rCBF measurements (H215O PET) were performed at rest and during a right hand movement. Order of pharmacological conditions and rest/movement were randomized. Statistical parametric mapping (SPM2) was used. Significance was set at Z score >3.2 ($P < 0.01$). As control group, ten age matched volunteers (66 ± 9 years) were included.

Results: In MSA patients, levodopa challenge did not modify UPDRS motor scale and ICARS scale. By contrast, PD patients were significantly improved by levodopa. In MSA patients, there was a severe hypoactivation in bilateral cerebellum during motor task in OFF condition, compared with controls. Levodopa did not induce any motor overactivity but there was a significant deactivation in anterior cingulate. In PD patients, motor task induced hypoactivation in supplementary motor area and hyperactivation in cerebellum in OFF condition, compared with controls. Levodopa significantly increased motor activation in ipsilateral cerebellum.

Conclusion: For movement, PD patients recruited cerebellar circuit and levodopa improved it. In MSA, there was no cerebellar activation during motor movement. Levodopa did not restore cerebellar activation and deactivated frontal areas. Such results suggested different levodopa effect on motor networks in MSA and PD.

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Behavioural and peripheral effects of a 2-week bupropion administration: a placebo and methylphenidate controlled, randomized, double-blind clinical study (NCT00285155)

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Introduction: Bupropion is largely used as an antidepressive and smoking cessation therapy. Its chemical structure and biological mechanisms are closely related to those of amphetamine-like drugs. We thus evaluated the pharmacological similarities between bupropion and the amphetamine-like compound methylphenidate, after repeated administration in man. Sponsor: CHU Montpellier; Funding: French Ministry of Health.

Methods: Twelve young male volunteers completed this double-blind, placebo and methylphenidate controlled, cross-over study, after informed consent. Bupropion and methylphenidate were orally administered for a first half-dose-6-day period (150 and 10 mg respectively) followed by a full-dose-8-day period (300 and 20 mg respectively). Outcomes were assessed after one night partial sleep-deprivation, before and after treatment, and comprised subjective feelings (self-rating questionnaires), cognitive functions (neuropsychological test battery), vital signs, appetite (visual analogue scales after a standardized breakfast), food consumption (*ad libitum* test meal). Data are means ± SEM. Comparisons were performed by ANOVA.

Results: Bupropion, similarly as methylphenidate, decreased the score of asthenia (44.2 ± 3.1 and 41.9 ± 3.7 respectively vs. 53.0 ± 4.1 for placebo; $P < 0.05$), despite an impairment of sleep onset (-4.3 ± 3.3 and -1.9 ± 3.8 respectively vs. +7.5 ± 3.7; $P < 0.05$). Both bupropion and methylphenidate increased resting diastolic blood pressure (67.9 ± 1.2 and 65.7 ± 1.0 respectively vs. 62.5 ± 1.4 mmHg; $P < 0.05$), body temperature (36.5 ± 0.1 and 36.5 ± 0.1 vs. 36.3 ± 0.1°C; $P < 0.05$) and decreased body weight (-0.7 ± 0.2 and -0.6 ± 0.2 respectively vs. +0.2 ± 0.3 kg; $P < 0.05$). No significant change could be observed with either bupropion or methylphenidate on heart rate, cognitive functions, appetite and energy consumption.

Conclusion: Our results suggest that bupropion has amphetamine-like properties, which can be revealed after 2 weeks of treatment, and are comparable to those of methylphenidate.

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Chronic pain in Parkinson's disease

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Introduction: Few data are available on different aspects of pain in Parkinson's disease (PD).Most clinical surveys are relatively old and do not allow comparisons (as they are limited to single centres or single patient) and are using different definitions of chronic pain. The cross-sectional DoPaMiP study was conducted in 2005 in the Midi Pyrenees Region to describe the prevalence and clinical presentation of chronic pain in PD patients.

Methods: Totally 450 outpatients with PD (UKPDSBB) were examined as outpatients by 30 neurologists and 98 matched non-PD patients were recruited as controls at GPs outpatient clinics. All patients had full standardized clinical and neurological examination for PD (UPDRS) and chronic pain assessment (VAS, pain 'relationship' with PD) plus self-administered questionnaires (anxiety and depression [HADS scale], quality of life [PDQ 39]) and analgesic consumption. Statistical analysis was performed using SAS (version 8) and ANOVA.

Results: Chronic pain was twice more frequent in PD patients than in control patients (OR: 1.9–IC 5% [1.2–3.2]). Overall, 65% PD patients suffered from any kind of chronic pain (International Pain Society). 39% PD patients suffered from a chronic pain 'related' to PD, while 26% had chronic pain due to another cause. When comparing PD patients with no chronic pain, chronic pain unrelated to PD and chronic pain related to PD, the last group was younger, had a more severe and advanced disorder, had more frequent motor complications, received a higher dose of levodopa, had greater UPDRS axial scores and had more severe anxiety, depression and quality of life scores ($P < 0.001$). Only 39% of patients with PD chronic pain received an analgesic, even if pain intensity was high (EVA = 6.5). Paracetamol was the most frequently prescribed treatment (34%) followed by level II analgesics (10%) while the efficacy of such drugs has never been assessed in this condition. Analgesic self medication was more frequent when pain was not related to PD.

Conclusion: A large majority of PD patients suffer from chronic pain. Compared with non-PD chronic pain patients and no pain patients, PD chronic pain patients were younger, had a more severe and advanced disease with more frequent motor complications and greater daily dose of dopaminergic therapies. PD Chronic pain patients had more severe scores on depression, anxiety and QOL. PD chronic pain appeared to be an heterogeneous syndrome. The efficacy/safety profile of none of the prescribed analgesics has ever been appropriately assessed in PD patients. This needs to be further explored.

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Is hypocretin involved in stress-induced sleep alterations in mice?

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Introduction: Hypocretin (Hcrt), a hypothalamic neuropeptide, is known to activate cerebral structures involved in sleep regulation such as Raphe nuclei (RN). Conversely, RN serotonergic neurons inhibit the hypocretinergic system. Hcrt and serotonin are thus key neuromodulators of sleep/wake cycle which both exert a negative influence on REM sleep. Interestingly, compared with wild-type (WT) mice, serotonin transporter deficient (5-HTT–/–) mice exhibit modifications of sleep regulation with increased REM sleep at baseline and absence of REM sleep rebound after restraint stress. The aim of our study was to specify interactions between hypocretinergic and serotonergic systems and their potential involvement in stress-induced sleep modifications in 5-HTT–/– mutants vs. paired WT mice.

Methods: *In situ* hybridization coupled with immunocytochemistry, double immunostaining of pre-prohypocretin and c-fos, and radioimmunoassays of hcrt1 in anterior raphe were performed to assess the activity of hypocretinergic system under basal conditions and after immobilization stress (90 minutes) in 5-HTT–/– mutants compared with WT mice (same CD1 background). In addition, polysomnographic recordings allowed us to analyse mice response to the same immobilization stress and the effects of specific hypocretinergic receptor 1 (hcrtR1) blockade by SB-334867 (30 mg/kg, i.p. acute injection or before stress session) on sleep and stress response in WT and mutant mice.

Results: In WT mice, stress increased c-fos expression (same results with *in situ* hybridization and double immunostaining) showing activation of hypocretinergic neurons without modification of hypocretinergic tone (unchanged hcrt1 levels in RN and pre-prohypocretin mRNA levels in the lateral hypothalamus). Under basal conditions, 5-HTT–/– mice exhibited higher tissue levels of hcrt 1 in RN and no changes in pre-prohypocretin levels. Immobilization stress also activated hypocretinergic neurons in mutants and increased hcrt1 levels in RN. In absence of stress, acute hcrtR1 blockade by SB-334867did not significantly affect vigilance states in both WT and 5-HTT–/– mice. In contrast, SB-334867 administration prior to the stress session restored stress-induced REM sleep rebound in 5-HTT–/– mice, like that usually observed after stress in naïve WT mice.

Conclusion: Altogether, we showed an alteration of hypocretinergic neurotransmission in 5-HTT–/– mice and a stimulatory effect of stress on hypocretinergic neurons in both these mutants and paired WT mice. Our data support the idea that functional interactions exist between hypocretinergic and serotonergic systems under basal conditions and after immobilization stress. Accordingly, the hypocretin system appeared to play a role in stress-induced sleep alterations.

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Both risperidone and clozapine but not haloperidol nor chlorpromazine increase focal cerebral ischemia severity in rat

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Introduction: Whether so-called atypical or second generation anti-psychotic drugs are associated or not to an elevated risk of stroke remains under debate (1). In this context, both US and European Drug Agencies have provided some restriction of use, particularly in elderly patients suffering from brain degenerative pathology such as Alzheimer disease and in patients who also present vascular risk factors such as arterial hypertension or diabetes. Beyond the possible increase in stroke incidence associated to atypical anti-psychotic drug treatments, recent data also suggest that such treatments may also lead to an increased severity and worse outcome of ischemic stroke (2). The aim of the present study was to determine the impact of a chronic treatment with a first or a second generation anti-psychotic drug (haloperidol, chlorpromazine, risperidone, clozapine) on behaviour and cerebral ischemia in rat.

Methods: Male Wistar rats (230–240 g) were treated through oral route during 4 weeks with either placebo, haloperidol (2 mg/kg), chlorpromazine (20 mg/kg), risperidone (2 mg/kg) or clozapine (20 mg/kg). Afterwards, they were tested for behavioural changes (both motor activity and spatial memory) before to be subjected to a 60-minute focal cerebral ischemia using the right MCA occlusion method followed by a 24-hour reperfusion period. Post-treatment fasting blood glucose levels, weight and brain histological data were also evaluated.

Results: Before cerebral ischemia, spontaneous motor activity was increased in chlorpromazine-, risperidone- and clozapine-treated rats but not in haloperidol-treated animals while in the Y-maze, rats previously treated with clozapine spent a longer time in the new arm during the retention test. Brain infarct volumes were increased in both risperidone and clozapine treated groups while no significant effect was observed with haloperidol or chlorpromazine as compared with placebo (risperidone, 246 ± 28 mm^3; clozapine, 236 ± 33 mm^3; haloperidol, 187 ± 10 mm^3; chlorpromazine, 192 ± 24 mm^3; placebo, 154 ± 17 mm^3; ANOVA, $P < 0.05$). Moreover, both typical and atypical anti-psychotic treatments also induced a significant increase in blood glucose levels (placebo, 1.60 ± 0.05 g/L; haloperidol, 2.15 ± 0.12 g/L; chlorpromazine, 2.19 ± 0.15 g/L; risperidone, 2.18 ± 0.10 g/L; clozapine, 2.03 ± 0.12 g/L; ANOVA, $P < 0.05$). Conversely, only haloperidol-treated animals had a lesser weight increase.

Conclusion: These data suggest a possible deleterious effect of second generation anti-psychotic drugs on stroke severity. Further studies are in course to explore underlying mechanisms and to evaluate the role of vascular risk factors.

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Behavioural assessment of two new drugs (pregabalin and duloxetine) potentialy effective in neuropathic pain treatment

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Introduction: We developed two animal models of nociceptive sensory neuropathy induced by repeated or acute administration of oxaliplatin in which treated animals reproduce the characteristic pain symptoms observed in cold conditions in patients treated by oxaliplatin for metastatic colorectal cancer. The aim of this study was to assess their pharmacological relevance in order to develop clinical trials.

Methods: To induce the chronic neurotoxicity model, treatment modality was a 2 mg/kg oxaliplatin dose (iv.), twice a week for 4 weeks (cumulative dose = 16 mg/kg). Concerning the acute model, a single injection (ip.) at 6 mg/kg has been previously validated. Behavioural sign used to assess nociceptive threshold was cold thermal allodynia, using the tail immersion test in cold water (10°C). Potential analgesic drugs assessed were single administration of pregabalin (2–10–100 mg/kg, i.v.) and duloxetine (3–10–30 mg/kg, i.p.), two new drugs potentially interesting in neuropathic pain treatment.

Results: Pregabalin, at lowest dose, induced a significant non-dose-dependent anti-allodynic effect, with a maximal effect at 150 min (+ 351%, $P < 0.001$ and + 187%, $P < 0.05$) in the chronic and acute models respectively. High-doses (10 and 100 mg/kg) did not induce significant effect on nociceptive thresholds. High-doses of duloxetine (10 and 30 mg/kg) displayed a significant dose–dependent antinociceptive effect at 105 min (+389%, $P < 0.001$ and +223%, $P < 0.01$) in the chronic and acute models respectively. When the effects of the more effective doses used in these models are compared, the effectiveness of the active tested drugs is as follow (previous studies): magnésium > venlafaxine = duloxetine > gabapentin > carbamazepin < pregabalin for the acute model and magnesium = venlafaxine > clomipramine > duloxetine > pregabalin > gabapentin < carbamazepin for the chronic model.

Conclusion: According to this study, pregabalin and duloxetine may be a alternative choice to treat acute and chronic sensitive disorders induced by oxaliplatin and need to be evaluated in clinical trials. Pregabalin seems to be more effective in chronic than acute model, suggesting different neurotoxic mechanisms between these two models.

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Effect of benzodiazepinic ligands on the anxiolytic-like activity of antidepressants in the four-plate test in mice

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Introduction: Benzodiazepines (BZDs) remain the first choice drugs for the treatment of anxiety disorders but these compounds involve several side effects (withdrawal symptoms, physiological dependence and sedation). More recently the specific serotonin reuptake inhibitors (SSRIs) have proved their efficacy in the treatment of anxiety disorders in humans. Precedent studies have demonstrated that the SSRIs and SNRIs possessed an anxiolytic-like effect in the four-plate test (FPT). The FPT is an animal model of anxiety in which exploration of novel surrounding is suppressed by the delivery of mild electric foot shock contingent to the quadrant crossing. The anxiolytic-like effect of these antidepressants seems to be mediated by 5-HT$_2$ receptors. Recently, we have demonstrated the implication of GABA system in the anxiolytic-like effect of DOI (a 5-HT$_{2A/2C}$ receptor agonist). The purpose of the present study was to examine the effect of four antidepressants (two selective serotonin reuptake inhibitors, paroxetine and citalopram; two serotonin and noradrenaline reuptake inhibitors, venlafaxine and milnacipran) and benzodiazepine ligands co-administration in the FPT. We have co-administered paroxetine, citalopram, venlafaxine and milnacipran with benzodiazepine receptor agonists (alprazolam and diazepam) and benzodiazepine receptor antagonist (flumazenil) in the FPT.

Methods: In the first time, the sub-active doses of antidepressants (ADs) were co-administered with inactive doses of benzodiazepine receptor agonists. In the second time, the active doses of ADs were co-administered with inactive doses of benzodiazepine receptor antagonist in the FPT. The benzodiazepine ligands were administered 45 min before the test and ADs 30 min before the test.
Results: Alprazolam (0.03 and 0.125 mg/kg) and diazepam (0.25 and 0.5 mg/kg) potentiated the anxiolytic-like effect of citalopram (0.5 and 1 mg/kg), paroxetine (0.25 and 0.5 mg/kg), venlafaxine (0.5 and 1 mg/kg) and milnacipran (1 and 2 mg/kg). Flumazenil (2 and 8 mg/kg) inhibited the anxiolytic-like effect of milnacipran (8 mg/kg), when only flumazenil (8 mg/kg) inhibited the anxiolytic-like effect of venlafaxine (8 mg/kg). Only, flumazenil (8 mg/kg) decreased anti-punishment effect of paroxetine (4 mg/kg). Flumazenil (8 and 2 mg/kg) did not inhibit the anxiolytic-like effect of citalopram (8 mg/kg).
Conclusion: Depending on ADs activity on noradrenergic system, their anxiolytic-like effects in the FPT in mice are differently influencing by benzodiazepine ligands.

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Transient protective effect of sevoflurane-induced pre-conditioning on focal cerebral ischemia in rats

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Introduction: Volatile anaesthetics possess direct but transient neuroprotective effects during ischemic cerebral injury probably mediated by a delayed development of apoptosis. We have previously shown that pre-conditioning with sevoflurane induced early neuroprotection against cerebral ischemia. This works aims to study the duration of sevoflurane-induced pre-conditioning and the role of apoptosis in this pre-conditioning.
Methods: Male Wistar rats (250 g; $n = 19$) were randomly allocated to control (C; $n = 9$) and pre-conditioning (P; $n = 10$) groups. Animals in both groups were anaesthetized with 3.0 vol% sevoflurane and subjected to transient middle cerebral artery occlusion (MCAO) by use of the intraluminal filament model. The animals were allowed to awaken and after 60 min of focal ischemia the filament was removed. In the pre-conditioning group, animals were exposed to 2.7 vol% sevoflurane (45 min) discontinued 60 min before MCAO. Neurological function (Bedersen's score) and infarct volumes (Nissl-staining) were determined after animals were killed at 14 days (C, $n = 9$; P, $n = 10$) after ischemia. Detection of DNA fragmentation by TUNEL staining and caspase-3 positive neurons were detected by immunolabelling.
Results: After a recovery period of 14 days in both groups none animal presented severe neurological deficits and infarct volumes in control and pre-conditioning groups were not significantly different (Day 14: C: 140.9 ± 25. vs. P: 148.1 ± 54.0 mm^3). The number of caspase-3 positive neurons was (Day 1: C: 20.0 ± 15 vs. P: 6.8 ± 1.2; Day 3: C: 49.8 ± 31.7 vs. P: 11.1 ± 6.9; Day 7: C: 40.33 ± 7.57 vs. P: 16.5 ± 7.1; Day 14: C: 4.8 ± 2.9 vs. P: 10.3 ± 2.6). The number of TUNEL positive cells was (Day 1: C: 10.8 ± 1.1 vs. P: 12.6 ± 0.3; Day 3: C: 20.2 ± 2.8 vs. P: 14.5 ± 0.7; Day 7: C: 6.9 ± 3.5 vs. P: 3.3 ± 1.8; Day 14: C: 2.8 ± 1.0 vs. P: 3.4 ± 0.7).
Conclusion: Sevoflurane-pre-conditioning only induced a transient protection against cerebral ischemia and delayed but not suppressed ischemia-induced apoptosis.

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CGRP- and 5-HT7- receptors, new targets for differential alleviation of cephalic vs. extracephalic neuropathic pain?

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Introduction: Neuropathic pain at cephalic level is particularly difficult to treat, and there is urgent need to develop really effective therapies. We previously found (Kayser et al., 2002) that antimigraine drugs, triptans, have some efficacy to reduce allodynia and hyperalgesia in a rat model of cephalic neuropathic pain. In addition to triptans, which act as 5-$HT_{1B/1D}$ receptor agonists, antagonists at CGRP- and 5-HT_7- receptors have been claimed to exert antimigraine effects. This led us to investigate whether these drugs may also have anti-allodynic/anti-hyperalgesic effects in rats suffering from cephalic neuropathic pain. For comparison, experiments were also performed on rats suffering from extracephalic neuropathic pain because previous studies showed that triptans are ineffective in these animals.
Methods: Adult male Sprague–Dawley rats underwent chronic unilateral injury (i.e. Loose ligatures) of the sciatic (extracephalic model) or the infraorbital (cephalic model) nerve. Mechanical hyperalgesia and allodynia were measured using Ugo Basile analgesimeter and von Frey filaments, respectively. Nociceptive reaction thresholds were markedly reduced 14 days after ligature of the sciatic nerve (paw withdrawal: 136 ± 3 g, vocalization: 274 ± 8 g, vs. 184 ± 10 g and 405 ± 16 g pre-operatively, respectively) or the infraorbital nerve (0.34 ± 0.05 g vs. ≥ 12.0 g pre-operatively). At that time, CGRP- or 5-HT_7- receptor ligands were administered, and reaction thresholds measured at regular time intervals.
Results: Acute 5-HT_7 receptor blockade (SB-269970, 3 mg/kg i.p.) partially reversed hyperalgesia in rats with sciatic nerve ligature (only on vocalization, 45–75 min after administration), but was ineffective in rats with infraorbital nerve ligature. In contrast, 5-HT_7 receptor stimulation (AS-19, 10 mg/kg s.c.) exerted strong and long-lasting (5 h, infraorbital nerve ligature; more than 18 hours, sciatic nerve ligature) antiallodynic effects, which could be prevented by pre-treatment with SB-269970. The CGRP receptor antagonist, BIBN 4096 (0.3 mg/kg i.v.), was marginally active in rats with sciatic nerve ligature, but markedly attenuated the hyper-responsiveness to mechanical stimulation of the face in rats with infraorbital nerve ligature (reaction threshold: ≈4 g, 2–4 h after BIBN 4096 administration).
Conclusion: These results support the idea that, depending on its cephalic/extracephalic location, neuropathic pain may respond differently to potentially analgesic drugs. Furthermore, they should promote studies aimed at assessing the clinical efficacy of CGRP receptor antagonists and 5-HT_7 receptor agonists to relieve neuropathic pain of trigeminal origin.

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Comparative effects of the dopaminergic agonists Piribedil and Bromocriptine in three different memory paradigms in rodents

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Introduction: The potential memory-enhancing properties of two dopamine agonists, Piribedil and Bromocriptine were evaluated in rodents in three experiments.
Methods: For the object recognition experiment male Sprague–Dawley rats were used, for the two radial maze experiments male C57Bl/6 mice were used (aged mice were 20–23 months old). Piribedil was used at 1 and 10 mg/kg s.c and Bromocriptine at 5 mg/kg s.c.
Results: While Piribedil (10 mg/kg) and Bromocriptine equally enhanced spontaneous object recognition in young adult rats (experiment A), only Piribedil displayed beneficial effects against ageing-related memory impairments in two radial-maze experiments in mice. First (experiment B), a two-stage paradigm of spatial discrimination was used to assess relational/declarative memory in aged mice. In stage 1, all groups learnt the constant location of food among six arms (three baited, three non-baited) by being repeatedly presented with each arm separately. In stage 2, mice were challenged with novel presentations, the arms being either combined into pairs of opposite valence ('two-choice' discrimination), or opened all six together ('six-choice' discrimination). All aged groups preferentially visited baited arms in 'six-choice' tests while only Piribedil (1 or 10 mg/kg) groups did so in 'two-choice' tests, previously demonstrated to be critical tests for relational/declarative memory. Hence Piribedil alleviated the relational/declarative memory failure seen in aged mice.
In a novel working memory task (experiment C), Vehicle- or Bromocriptine-treated aged mice displayed, compared with (Vehicle) younger controls, a severe and persistent deficit in short-term retention of successive arm-visits, performing close to chance whichever the retention interval. Performances of Piribedil (10 mg/kg) group remarkably improved across testing-days and reached young adults' level.
Conclusion: The restoration of specific mnemonic impairments in mice highlights the potential interest of Piribedil in treating cognitive symptoms of Parkinson disease.

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Consumption of cannabis among subjects with history of abuse/dependence or under an opiate maintenance therapy: OPPIDUM data in 2004 and main trends since 2000

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Introduction: Since these last years, the consumption of cannabis has increased. Nevertheless there are few data about these characteristics. The consumption of cannabis in 2004 has been studied using OPPIDUM program. The main trends have been assessed since 2000.
Methods: The Oppidum Program (Observation of illegal drugs and misuse of psychotropic medications), a multicentric survey, annually surveys drug dependent subjects attending specialized care centres throughout France. Data were collected by questionnaire on sociodemographic variables and drug use during the preceding week.
Results: During October 2004, 3373 subjects were included. 41% ($n = 1393$) of them used cannabis, and among them, 25% ($n = 342$) used only cannabis and 75% ($n = 1051$) used cannabis with other psychoactive substances (SPA). Subjects who consumed only cannabis are younger, with a better economic situation in comparison with the others consumers of cannabis. This study underline the misuse of cannabis which is more important in the sub-group « only cannabis » (38% of suffering when stop cannabis, 65% of daily consumption, 37% of alcohol concomitant, 71% of description of abuse or dependence witch cannabis).
Conclusion: This study underlines the presence of a sub-group « consumer only of cannabis » in the drug dependent patients recruited by specialized care centres. This sub-group had specific economics characteristics and a specific consumption of cannabis. These data confirm the need of specific consultation in place for this population as planned in the governmental plan of fight against illicit drug, tobacco, alcohol 2004–2008.

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Triggering factors for one-trial tolerance in the four-plate test retest in mice

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Introduction: One-trial tolerance to benzodiazepines has been described with rodents in some tests of anxiety, e.g. in the elevated plus-maze, the light/dark transition. This loss of effect of drugs observed during trial 2 on previously undrugged mice is of great interest to study structures implicated in the anxiolytic-like effect of benzodiazepines and the network implicated during such stimulations. In the present study, we have considered the behaviour of mice through two drives: exploration and fear, in order to discover the main causes of the loss of effect of diazepam in four-plate test-retest. To achieve this, we have used different test–retest protocols during trial 1, implying presence or absence of electric punishments, spatial modifications, or exposure to another anxiety test as trial 1 (elevated plus-maze). Altogether, these modifications of test–retest would help us to describe better the loss of effect and to link it with one-trial tolerance to benzodiazepines. In parallel, we have studied the effect of DOI, a selective 5-HT $2_{A/C}$ agonist, which keep its anxiolytic-like effect during trial 2.
Methods: Naïve Swiss mice were exposed to four-plate test with or without electric punishments (Experiment 1, 2) or to a four-plate test with spatial modifications and electric punishments (Experiment 3) or to elevated plus-maze (Experiment 4). During the second exposure, 24 hours later, mice were injected i.p. With drugs and submitted to a four-plate test with electric punishments.
Results: Removing punishments in trial 1 is not sufficient to counteract the loss of effect of diazepam, but a spatial modification of the aversive environment in trial 1 restores its anxiolytic-like effect during trial 2. Exposure to elevated plus-maze then four-plate test does not trigger a loss of effect of diazepam.

Conclusion: The loss of effect observed with diazepam during trial 2 with four-plate test can be considered as a one-trial tolerance phenomenon. Fear of the punishment and exploratory drives are both implicated in this anxiety test, but they do not have the same weight when considering test–retest results. Punishment is not the triggering factor of this loss of drug effects, it acts only as a potentiator; whilst knowledge of the environment seems to be the main reason in the appearance of one-trial tolerance to benzodiazepines. Four-plate test may represent a good tool to study one-trial tolerance to benzodiazepines in a test of anxiety and to dissect mechanisms of action of this compounds; because the presence of two opposite drives create a situation of risk assessment deeply implied in anxiety. Furthermore, punishments represent an instrument to sensitize the results obtained in four-plate test.

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Clinics and pre-clinics studies of the endogenous and exogenous concentrations of GHB in various biological matrices

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Introduction: Neurotransmitter and neuromodulator of the GABA-ergique system, concentration of endogenous gamma-hydroxy-butyrate (GHB) was increase after death. *In vivo*, GABA is converted to GHB by the action of succinic semialdehyde reductase, and from 1.4 Butanediol (1.4 BD) by the action of alcohol deshydrogenase in the central nervous system. GHB is also produced from Gamma-Butyrolactone (GBL) by the action of a peripheral lactonase.
Methods: The first aim of this work was to develop a reproducible, sensitive and specific method to determine levels of GHB and all its metabolites in different samples: on pre-clinical and forensics samples. However, studying GHB kinetics after deaths requires the analysis of numerous tissues including those of the central nervous system and also peripheral ones.
The second aim of this work was to evaluate variability of endogenous GHB concentration in different population and determinate physiological concentrations of GHB in whole blood.
Lastly, an ambitious clinical protocol evaluates the incorporation of GHB in hair matrix. The aim of this work was to evaluate the incorporation of GHB in hair and determine if this biological matrix should be used to identify voluntary or involuntary administration of GHB in different forensics expertises.
Results: Different relation between variation of post-mortem GHB concentration and post-mortem interval presents significant correlation only for the animal model and not for human expertises.
Moreover, this study confirms the real difficulty to identify exogenous GHB administration in different biological matrices.
Conclusion: The biological interpretation of GHB concentrations was very difficult to realize: especially when samples were taking away out remotely of administration.

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Minocycline exerts anti-edematous and anti-inflammatory effects in a model of diffuse head injury

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Introduction: Minocycline, a semi-synthetic second-generation tetracycline, has shown to exert anti-inflammatory and neuroprotective effects, unrelated to its anti-microbial action, in several animal models of neurodegenerative diseases and acute brain injuries. However, the effect of minocycline on the consequences of traumatic brain injury (TBI) is still not fully investigated. One of the mechanisms of the neuroprotective action of minocycline is related to its inhibitory effect on microglial activation and the p38 mitogen-activated protein kinase (MAPK) activation. Therefore, our aim was to study the effects of minocycline in a murine model of diffuse head injury, which mimics severe TBI.
Methods: Closed head injury, model of diffuse head injury, was performed in mice. In order to design an appropriate treatment protocol, time courses of microglial activation and brain IL-1β levels were first evaluated by CD11b immunohistochemistry and ELISA, respectively. Activation of p38 MAPK was studied by Western blotting. Minocycline was administered twice, 5 min (90 mg/kg, i.p.) and 6 h (45 mg/kg, i.p.) following TBI. Cerebral oedema and microglial activation were evaluated at 24 h, whereas brain IL-1β levels and p38 MAPK activation were measured at 6 h post-TBI where IL-1β levels reached its maximum.
Results: Diffuse head injury leaded to several events, (1) acute and persistent CD11b immunoreactivity restricted to brain lesion area up to 72 h, (2) acute and transient elevation of brain IL-1β levels with a maximum 6 h after TBI compared with naïve animals (14.5 ± 4.15 pg/mg protein vs. 1.8 ± 0.06 pg/mg protein, $P < 0.001$), (3) an absence of p38 MAPK activation from 1 h to 48 h post-TBI and finally (4) a cerebral edema formation of 2% at 24 h post-TBI. Treatment with minocycline markedly decreased the CD11b immunoreactivity in injured mice as well as the TBI-induced IL-1β elevation compared with vehicle-treated TBI mice (16.2 ± 1.4 pg/mg protein vs. 26.7 ± 3.08 pg/mg protein, $P < 0.01$). However, the treatment had no effect on the basal level of p38 MAPK activation. Finally, treatment with minocycline led to a reduction of TBI-induced cerebral edema by 50% ($P < 0.01$).
Conclusion: This study provides the first evidence showing the anti-oedematous and anti-inflammatory effects of minocycline in a model of diffuse brain injury. The beneficial effects of minocycline in our model seem to be independent of p38 MAPK activation. Finally, our data provide a rational to test minocycline, at least as anti-oedematous strategy, in severe head injury patients.

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MRI investigation of brain lesions after prenatal hypoxia or hypoxia-ischemia

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Introduction: Despite considerable improvements in neonatalogy and obstetrics treatments, and in spite of a spectacular decrease of neonatal morbidity and mortality, the prevalence of neurologic disabilities of perinatal origin has remained unchanged in the last years. A noxious intra-uterine environment such as hypoxia or hypoxia-ischemia occurring during embryogenesis alters the neural development and thus produces various neurological disorders observed postnatally (e.g. mental retardation, seizures, epilepsy).
Our goal was to develop a MRI approach to investigate the impact of pre-natal hypoxia-ischemia or hypoxia on the appearance of brain lesions (atrophy, necrosis or haemorrhage) and to assess *in vivo*, non-invasively, the spatial distribution, extension and evolution of lesions.
Methods: Hypoxia-ischemia was induced by ligaturing vessels near the lower end of the uterine horn in the pregnant female rat on embryonic day 17. Hypoxia was caused by having the dam reside in a hypoxic normobaric chamber during the gestation. Fast T2-weighted RARE sequence MRI (on a 4.7-T Bruker Biospec imager) was used on hypoxic pups ($n < 40$) and controls ($n < 40$) at different development stages.
Results: Thanks to MRI, we were able to measure a large white matter atrophy after both hypoxia-ischemia and hypoxia alone. White matter thicknesses of affected neonate brains were significantly different from the controls, at all stages. Such pre-natal insults thus alter the normal growth of the white matter.
We observed necrotic and haemorrhagic lesions, showing as signal alterations in the intraventricular area and/or in the brain parenchyma of several hypoxic-ischemic or hypoxic animals. No such features were seen in controls. Highly severe multifocal necrotic lesions coupled with haemorrhage were found in the parenchyma of a number of pups, bearing an unfavourable prognosis.
MRI allows us to witness lesion evolution *in vivo*. Haemorrhagic lesions initially appear as a hyperintensity in T2-weighted images at 4.7 Tesla, and then generally evolve with time into a hypo-intensity. Thus, we can say that the hypointensity corresponds to the initial, *in utero* haemorrhagic injury and that the hypersignal surrounded by hyposignal at its periphery corresponds to a haemorrhagic necrosis.
Conclusion: In this innovative application of T2-weighted MRI in the young small animal, we were able to characterize the brain lesions (type, severity, topography and progress) induced in two neonatal rat models (hypoxic-ischemic and hypoxic). Namely, we measured a white matter atrophy, located necrotic and/or haemorrhagic lesions and assessed their evolution.

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Acute neuroprotective effect of PPAR-α activator in a cerebral ischemia-reperfusion model: leucocyte-vessel interaction

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Introduction: In the course of cerebral ischemia peroxisome proliferator-activated receptor-α activation induces preventive neuroprotective effect in particular by the prevention of ischemia-induced leucocyte protein adhesion expression in cerebral vessels. The aim of this study is to bring to the fore in a cerebral ischemia-reperfusion model: (i) the neuroprotective effect of PPAR-α post-ischemic activation; (ii) the involvement of vascular wall and leucocyte activation in this neuroprotective effect.
Methods: Mice were subjected to a 30 minutes middle cerebral artery occlusion or sham surgery. Vehicle or fenofibrate (50 mg/kg/day), a PPAR-α activator, was administered by gavage twice a day during 72 hours after onset of reperfusion. During this treatment period cerebral microcirculation was studied by leucocyte activation and vascular reactivity analysis thanks to intravital microscopy. The infarct volume was determined at 72 hours by histomorphometry.
Results: PPAR-α activation induced a significant decrease in infarct volume (23.4 ± 4.8 mm^3) in comparison to vehicle-treated animals (40.0 ± 3.6 mm^3, $P < 0.01$). In parallel fenofibrate treatment was associated to post-ischemic leucocyte activation diminution at arteriolar and venular level. This effect was observed on leucocyte rolling and adhesion without modulating shear rate in mice cerebral microcirculatory. Finally fenofibrate led to preserve partially endothelium-dependant arteriolar vasodilatation (19.2 ± 2.3% in fenofibrate-treated group vs. 12.8. ± 1.7% in vehicle-treated group, $P < 0.05$).
Conclusion: Our study show post-ischemic treatment by fenofibrate induce neuroprotective effect in parallel to leucocyte activation decrease and vascular reactivity preservation in a cerebral ischemia-reperfusion model. These results suggest the couple leucocyte-endothelium could be a PPAR-α activation target which could contribute to his beneficial effect in the course of cerebral ischemia.

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Effect of PJ34, a poly (ADP-ribose) polymerase inhibitor, on intracerebral haemorrhage, motor functions and infarct volume after permanent focal cerebral ischemia in mice

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Introduction: Intracerebral haemorrhage occurs in 15 to 43% of patients suffering cerebral ischemia (1). Recent experimental studies demonstrate that poly (ADP-ribose) polymerase (PARP) enhances the expression of the brain matrix metalloproteinase-9 in cerebral ischemia (2). Matrix metalloproteinase-9 is known to degrade the basal membrane components of blood vessels and to contribute to the development of intracerebral haemorrhage after brain ischemia. In this context we assessed the effect of PJ34 (N- (6-oxo-5,6-dihydro-phenanthridin-2-yl) -N-N-dimethylacetamide), a PARP inhibitor, on intracerebral haemorrhage subsequent to ischemia.
Methods: Ischemia was induced by a permanent intravascular occlusion of the left middle cerebral artery in male Swiss mice anaesthetized with i.p. Ketamine (50 mg/kg) and xylazine hydrochloride (6 mg/kg). Sham-operated animals underwent all the surgery except the artery occlusion. PJ34 (6.25 mg/kg; dissolved in saline) was given i.p. Just before and 4 h after ischemia. After 48 h of ischemia, animals were attributed a motor function score (1 to 11, the highest the score, the highest the deficit). Animals were then killed and cerebral tissue removed for the evaluation of intracerebral haemorrhage and infarct volume. Intracerebral haemorrhage, defined as petechiae or hematoma, were counted visually on 50 μm-thick coronal brain slices every 500 μm interval.
Results: Saline-treated ischemic mice showed a significant increase in the score of petechiae and haematoma (29 ± 4 and 46 ± 12 respectively) compared with sham-operated mice (5 ± 2 and 4 ± 3 respectively), 48 h after the onset of

ischemia. Treatment with PJ34 significantly reduced both the score of petechiae (–38%) and hematoma (–69%) compared with that of saline-treated ischemic animals ($P < 0.05$).
Ischemic animals exhibited a significant increase in the motor function score compared with sham-operated mice (7 ± 1 vs. 1 ± 1, $P < 0.01$), indicating a motor deficit. PJ34 improved motor functions (3 ± 1, $P < 0.05$ vs. saline-treated ischemic mice).
PJ34 also significantly reduced the infarct volume (–30%, $P < 0.01$).
Conclusion: The present study indicates that PARP activation may play a major role in the development of intracerebral haemorrhage subsequent to brain ischemia.

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Primary haemostasis impairment as a surrogate biomarker of severity in aortic stenosis
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Introduction: Stenosis-induced high shear stress is considered as a major determinant of von Willebrand Factor impairment in patients with valvular aortic stenosis (AS). Previous studies have demonstrated that primary haemostasis abnormalities correlate with the severity of AS.
Accordingly, we sought to evaluate the diagnostic value of this haemostatic impairment as a surrogate biomarker of severity in AS.
Methods: Sixty consecutive patients (71 ± 15 years, 26 males) prospectively underwent Doppler echocardiography as well as blood sampling to evaluate primary haemostasis abnormalities. None patient received clopidogrel or ticlopidine.
Results: Left ventricular ejection fraction averaged $58 \pm 12\%$, aortic valve area was 0.94 ± 0.3 cm^2 and mean transvalvular pressure gradient was 37 ± 18 mmHg. Maximal transvalvular velocity ranged from 2 to 6.5 m/s and 24 patients had a maximal transvalvular velocity higher than 4 m/s that has previously been associated with worse prognosis in the setting of AS. Shear stress induced platelet adhesion was prolonged (PFA-100® ADP ≥ 114 s) in 39 patients. The PFA-100® ADP did correlate with maximal transvalvular velocity ($r = 0.54$, $P < 0.0001$), mean transvalvular gradient ($r = 0.53$, $P < 0.0001$) and aortic valve area ($r = –0.35$, $P = 0.005$). Sensitivity and specificity of a PFA-100® ADP ≥ 114 s to predict maximal transvalvular velocity more than 4 m/s were 92 and 53% respectively (area under curve (AUC) 76%, $P < 0.0001$).
Conclusion: Baseline obstruction leads to primary haemostasis impairment that may be considered as a surrogate biomarker of severity in the setting of aortic stenosis. Further studies are needed to evaluate the prognostic value of PFA-100® ADP ≥ 114 s in AS.

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Short-term blood pressure control in patients with cardiac transplantation
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Introduction: Cardiac component of the baroreflex is responsible for attenuation of blood pressure fluctuations in the range of frequencies 0.04–0.15 Hz (low frequency, LF). Deinervation of the heart in patients after orthotopic cardiac transplantation (OTC) eliminates this mechanism.
The aim of the study was to analyse short-term blood pressure control and blood pressure variability in the range of frequencies 0.04–0.15 Hz in patients after OTC and in healthy controls.
Methods: We examined 7 patients (age 55.7 ± 9.7 years) after 2–8 years after cardiac transplantation. ECG, blood pressure (BP) and thoracic impedance were recorded beat-by-beat during 20 minutes (Task Force Monitor, CNSystem, Austria) in supine position during spontaneous breathing and breathing controlled by metronome (5 min, 0.33 Hz). The results were compared with the results of examination of the group of seven healthy subjects (C) of similar age (50.0 ± 2.8 years).
Results: Both groups did not differ (OCT vs. C, mean ± SD) in systolic (119.4 ± 11.8 vs. 124.9 ± 11.0 mmHg) and diastolic BP (80.6 ± 10.3 vs. 85.7 ± 8.3 mmHg), in stroke volume index (32.6 ± 8.9 vs. 39.9 ± 6.6 mL/m^2), in cardiac index (2.54 ± 0.55 vs. 2.90 ± 0.58 l/min m^2) and in total peripheral resistance index (2863 ± 552 vs. 2754 ± 845 dyn s m^2/cm^5). On the other hand, heart rate variability spectra (ms^2) were decreased in OTC (LF heart rate variability: 8.43 ± 12.09 vs.164.29 ± 171, $P < 0.01$). No difference was seen in diastolic BP variability spectra (mmHg^2): LF dBP (5.00 ± 8.82 vs.3.10 ± 1.94, n.s.).
Conclusion: It is concluded that blood pressure variability in the range of frequencies 0.04–0.15 Hz in patients after OTC is unchanged in patients after OTC despite of deinervation of the heart in comparison with healthy controls.
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Cardiac-related oscillations of renal sympathetic nerve activity in conscious rats: a new insight into sympathetic baroreflex function
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Introduction: The classical approach for assessing the baroreflex control of sympathetic nerve activity is the so-called pharmacological method. However, due to its poor temporal resolution, this method is inadequate for the continuous assessment of the sympathetic baroreflex sensitivity. In rats, renal sympathetic nerve activity shows a strong rhythmicity at heart rate frequency which is entirely mediated by the arterial baroreceptor reflex. In addition, the baroreflex negative feedback loop is virtually opened at the frequency of the heart beat (5–8 Hz in rats) because oscillations of sympathetic nerve activity are not translated into arterial pressure fluctuations at this frequency. Therefore, the present study examined whether the gain of the transfer function relating the cardiac-related oscillation of renal nerve activity to the arterial pressure pulse might serve as a spontaneous index of sympathetic baroreflex sensitivity. The ability of the new index to predict buffering of arterial pressure fluctuations was examined and taken as an indication of its functional relevance.
Methods: Arterial pressure and renal nerve activity were simultaneously recorded in conscious freely behaving rats, either baroreceptor-intact (control, $n = 11$) or with partial (aortic) deinervation of baroreflex afferents ($n = 10$) in order to enlarge the range of variation of the sympathetic baroreflex sensitivity. The transfer gain at heart rate frequency was calculated over 58 adjacent 61-s periods (each segmented into 10.2-s periods). Only gain values that were associated with a significant coherence (demonstrating a reliable linear relationship) were included in the final calculations.
Results: Aortic baroreceptor deinervation decreased the percentage occurrence of significant coherence values (56 ± 10 vs. $90 \pm 3\%$; $P = 0.007$) as well as the mean value of the transfer gain (1.48 ± 0.22 vs. 2.39 ± 0.13 normalized units/mmHg; $P = 0.005$). In the pooled study sample, the 1-h mean transfer gain was correlated with the sympathetic baroreflex sensitivity estimated by the vasoactive drug injection technique ($R = 0.75$; $P < 0.0001$) and was inversely related to both time- (standard deviation; $R = –0.74$; $P = 0.0001$) and frequency-domain (total spectral power; $R = –0.81$; $P < 0.0001$) indices of arterial pressure variability. In control rats, the transfer gain exhibited large fluctuations (variation coefficient = $34 \pm 3\%$) that were not consistently related to changes in the mean level of arterial pressure, heart rate or renal nerve activity.
Conclusion: The transfer function method provides a continuous, functionally relevant index of the sympathetic baroreflex sensitivity, and suggests that the latter fluctuates widely over time.

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Activation of AMP kinase alpha1 subunit induces aortic vasorelaxation in mice
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Introduction: Vasorelaxation is a vital mechanism of systemic blood flow regulation that occurs during periods of increased energy demand. The AMP-dependent protein kinase (AMPK) is a serine/threonine kinase activated by conditions that increase the AMP-to-ATP ratio, such as exercise and metabolic stress. We hypothesized that AMPK could trigger vasodilatation and participate in blood flow regulation.
Methods: Rings of thoracic aorta were isolated from C57BL6 mice and mice deficient in the AMPK catalytic α1 (AMPKα1$^{-/-}$) or α2 (AMPKα2$^{-/-}$) subunit and their littermate controls, and mounted in an organ bath. Aortas were pre-constricted with phenylephrine (1 μM) and activation of AMPK was induced by addition of increasing concentrations of 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR).
Results: AICAR (0.1–3 mM) dose-dependently induced relaxation of pre-contracted C57BL6, AMPKα1$^{+/+}$ and α2$^{+/+}$ aorta ($P < 0.001$, $n < 5$ per group). This vasorelaxation was not inhibited by the addition of adenosine receptor antagonists. Moreover, when aortic rings were freed of endothelium by gentle rubbing, AICAR still induced aortic ring relaxation, suggesting a direct effect of AICAR on smooth muscle cells. When aortic rings were pre-treated with L-NMMA (30 μM) to inhibit nitric oxide synthase activity, AICAR still induced relaxation. Finally, AICAR-induced relaxation of aortic rings was completely abolished in AMPKα1$^{-/-}$ but not AMPKα2$^{-/-}$ mice.
Conclusion: Taken together, the results show that activation of AMPKα1 but not AMPKα2 is able to induce aortic relaxation in mice, in an endothelium and eNOS-independent manner.

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Platelet aggregation induced by ADP in rats *in vitro*
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Introduction: The blood platelets play a major role in haemostasis. The aim of our study was to investigate platelet aggregation in normal and obese rats. The effects of adenosine diphosphate on platelet aggregation was studied for three batches of male rats: normal animals (*Rattus norvegicus* and *Psammomys obesus*) maintained on standard diet and obese animals (*Psammomys obesus*) maintained on hypercalorific diet.
Methods: The blood is removed from the jugular vein. After centrifugation between 100 and 120 g during 10 minutes, the adenosine diphosphate is added to the plasma. Platelet aggregation is carried out using an APACT aggregometer. Intensity of aggregation is measured two minutes after adenosine diphospate addition.
Results: Results show that for *Rattus norvegicus*, the amounts of adenosine diphosphate for 2.5 μM, 1,25 μM and 0,6 μM induced an average aggregation intensity of 43, 24, and 5.5 mm respectively. For normal *Psammomys obesus* and with the same amounts of adenosine diphosphate we obtained an average intensity of 20.2, 22.5and 20.5mm. In the same way and for obese *Psammomys obesus*, the values of intensity were 40, 43, and 42 mm.
These preliminary results show that the intensity of platelet aggregation for *Rattus norvegicus* depends of adenosine diphosphate amount. For *Psamomys obesus*, the intensity of platelet aggregation does not change with the amount of adenosine diphosphate and was strongly elevated in obese animal.
Conclusion: Other research involving made diabetic sand rats will be conducted shortly to asses the effect of various therapeutic agents on platelet function. The sand rat *Psammomys obesus* appears to us as a potentially interesting model for investigation in diabetic syndrome and his effects on platelet function.

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Ultrasonic imaging of cerebral circulation in a model of ischemia-reperfusion in 7 day-old rat
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Introduction: Perinatal hypoxia and ischemia is a cerebrovascular event around the birth with pathological evidence of focal arterial infarction with an incidence of approximately one case for four thousand children. A model has been developed to study cellular and molecular events during cerebral ischemia; it combines occlusion

of the left middle cerebral artery with a transient occlusion of the common carotid artery. In this model, the size of the ischemic lesion is not constant. Thus, we developed a method to analyse the blood flow distribution in the main cerebral arteries, upstream and downstream the Willis circle using color-coded pulsed Doppler ultrasound imaging.
Methods: With a Vivid 7, GE Medical Systems *ultrasound* ®, Horten, Norway, equipped with a 12-MHz linear transducer, we measured in 10 rat pups, variations of peak systolic, end-diastolic and time-average mean blood flow velocities in the cerebral arteries, which occurred during experimental procedure (coagulation of the distal left middle cerebral artery – occlusion of the left common carotid artery – release of the occlusion).
Results: We evidenced the efficacy of collateral arterial supply through the Willis circle. Indeed, after coagulation of the distal left middle cerebral artery which enhanced a local distal arterial occlusion, and the additional occlusion of the left common carotid artery, which enhanced a dramatic hypoperfusion in the left internal carotid artery, we demonstrated that blood flow velocities only decreased by 35% in the left middle cerebral artery, while blood flow velocities increased in the right carotid artery by 57% and in the basilar trunk by 34%. After removal of the clip on the left common carotid artery, only three pups recovered a reperfusion of the left internal carotid artery.
Conclusion: Technical improvement of ultrasound device allows our new approach for study cerebral circulation in the rat pup. This method is a simple, accurate and currently available technique, which could be a valuable tool to assess circulatory changes in the cerebral vasculature in rat pup. This non-invasive method could be repeated allowing longitudinal survey. The monitoring of blood flows in cerebral arteries could thus help to quantify the effect of cerebral protectors therapy during experimental procedure of ischemia-reperfusion in rat pups.

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Estimation of the functional role of arterial pathways to the buttock circulation during treadmill walking in patients with claudication
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Introduction: The aim of the study was to estimate the functional contribution of the arterial inflow pathways to the pelvic circulation, during walking, in patients with stage 2 lower extremity arterial disease. Transcutaneous oxygen pressure (tcpO2) changes during exercise can be used to estimate the severity of regional blood flow impairment while walking.
Methods: Seventy patients with stable lower limb claudication were studied using a multi-variant linear regression model. The relationship between exercise-induced buttock tcpO2 changes, the ipsilateral calf tcpo2-changes and the arterial diameters of the pelvic arteriographic pathways were analazed.
Results: The ipsilateral hypogastric and lumbar pathway, as well as the ipsilateral calf tcpo2-changes were the only variables significantly related to buttock tcpO2-changes ($r = 0.47$; $P < 0.001$). Their normalized respective contribution to the regressive model was 39, 19 and 18% respectively. None of the contra-lateral hypogastric, mesenteric and sacral pathways or pathways stemming from the external iliac artery, showed significant correlation to buttock tcpO2-changes.
Conclusion: The ipsilateral hypogastric and ipsilateral lumbar pathways are the major pathways responsible for the functional buttock blood flow supply during walking. The role of contra-lateral hypogastric, inferior mesenteric and median sacral pathways and arteries distal to the internal iliac trunk is negligible in the normal or compensatory blood flow supply. Distal tcpo2 decrease at exercise aggravates proximal tcpO2 decrease, possibly through the occurrence of a 'steal phenomenon' of distal over proximal circulation during walking.

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Determinants of pulmonary artery pressure in valvular aortic stenosis
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Introduction: Some patients with valvular aortic stenosis (AS) present with pulmonary hypertension and high transtricuspid pressure gradient (TTG) that conveys to a poor prognosis.
This study aimed to determine 1) the relationship between the TTG and the clinical and biological profile of AS patients 2) the main determinants of the TTG in these patients.
Methods: Ninety-four patients (71 ± 13 years, 48% male) prospectively underwent Doppler echocardiography and blood sampling.
Results: Thirty-four per cent were symptomatic (defined as dyspnea related to heart failure, chest pain or syncope). Left ventricular ejection fraction (LVEF) ranged from 15 to 82%, aortic valve area (AVA) ranged from 0.21 to 1.66 cm^2 and the TTG ranged from 12 to 65 mmHg. The TTG was higher in symptomatic compared with asymptomatic patients (33 ± 12 vs. 24 ± 8 vs. $P = 0.002$). TTG correlated with log B-Natriuretic peptide, a marker of early cardiac failure ($r = 0.58$, $P < 0.0001$). By univariate analysis, TTG correlated with LVEF ($r = -0.28$, $P = 0.01$), left atrial (LA) surface ($r = -0.4$, $P < 0.0001$), the E/E' ratio (a non-invasive marker of LV end diastolic pressure) ($r = 0.26$, $P = 0.03$) and aortic valve area ($r = -0.41$, $P < 0.0001$). By contrast, the TTG did not correlate with mean and maximal transvalvular pressure gradients ($r = 0.15$, $P = 0.17$ and $r = 0.18$, $P = 0.11$ respectively). By multivariate analysis, independents echocardiographic determinants of TTG were LA surface ($P = 0.01$) and AVA ($P = 0.003$).
Conclusion: The TTG is associated with clinical and biological markers of heart failure in the setting of AS. Aortic valve area and left atrial size, an integrator of LV diastolic function, are the main determinants of TTG in AS. Further studies are needed to confirm these results.

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Cross-talk between the two thrombin receptors in human endothelial cells
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Introduction: The endothelial thrombomodulin is the high affinity receptor of thrombin. As recently shown, this endothelial receptor regulates not only coagulation, but also inflammation and cell proliferation. We previously demonstrated that thrombin binding to thrombomodulin mediates a specific outside-in signal to activate the endothelial nitric oxide synthase and the receptor tyrosine kinase of epidermal growth factor and to modulate the signalling of the low affinity receptor of thrombin, PAR-1 (*J. Biol .Chem.* 2005, 280: 35999). In the current study, we investigated the mechanism by which the two thrombin receptors, thrombomodulin and PAR-1, mediate transactivation of the epidermal growth factor receptor and the soluble tyrosine kinase Src.
Methods: Human umbilical vein endothelial cells are stimulated by thrombin or a mutant of thrombin that binds to the epidermal growth factor-like domain of thrombomodulin as thrombin does, but has no catalytic activity on the PAR-1. Phosphorylation of signalling proteins is assessed by Western blots. NO release at cell surface is detected by electrochemistry.
Results: Under 1 nM, thrombin stimulated the phosphorylation of phospholipase Cγ and above 1 nM, it activated the phospholipase Cβ with no phosphorylation of PLCγ, showing a strict regulation between the two thrombin receptors. The two distinct signalling pathways are associated with a biphasic activation of both endothelial nitric oxide synthase and cytosolic phospholipase A_2. In contrast, the phosphorylation of ERK1/2 and p38 directly increased with thrombin concentrations, suggesting that only one receptor is involved in the activation of mitogen activated protein kinases. Transactivation of the epidermal growth factor receptor is observed in response to the mutant trombin, the thrombin receptor activating peptide and high concentration of thrombin. The transactivation is associated with Src phosphorylation through a nitric oxide-dependent mechanism.
Conclusion: Though thrombomodulin and PAR-1 activate distinct signalling pathways, they both appear to mediate transactivation of the epidermal growth factor receptor through a common mechanism. We postulate that thrombin-activated metalloprotease may release the thrombomodulin ectodomain that contains a growth factor region thereby forming a soluble ligand for the epidermal growth factor receptor.

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AMP-activated protein kinase is involved in both NO- and EDHF-mediated endothelium-dependent relaxations to red wine polyphenols
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Introduction: Several epidemiological studies have shown that regular consumption of moderate amounts of wine, in particular red wine, is associated with a decreased total mortality due, in part, to a reduced risk of cardiovascular diseases. The protective effect has been attributable to polyphenols, which are potent vasodilators and have anti-thrombotic properties. Polyphenols have been shown to induce pronounced endothelium-dependent relaxations of arteries by causing the redox-sensitive PI3-kinase-dependent formation of nitric oxide (NO) and endothelium-derived hyperpolarizing factor (EDHF). The aim of the present study was to determine the role of the stress-activated AMP-activated protein kinase (AMPK) in the red wine polyphenols (RWPs) -induced endothelial formation of NO and EDHF.
Methods: Rings of aorta and mesenteric arteries from 12 to 14 weeks-old male Wistar rats, and of porcine coronary arteries were suspended in organ chambers for recording of changes in isometric tension. Cultured porcine coronary artery endothelial cells were used to study the phosphorylation level of endothelial NO synthase (eNOS) at serine 1179, and AMPK at the threonine 172 by Western blot analysis. RWPs were prepared from a French red wine from the southwest containing 2.9 g/L polyphenols expressed as Gallic acid equivalents.
Results: RWPs caused endothelium-dependent relaxations in rings from rat aorta and mesenteric artery, and in those from porcine coronary artery. NO-mediated relaxations to RWPs in aortic rings, and in coronary artery rings as assessed in the presence of indomethacin and charybdotoxin plus apamin, were inhibited by compound C (an inhibitor of AMPK). Compound C also reduced EDHF-mediated relaxations as assessed in the presence of indomethacin and N^G-nitro L-arginine in mesenteric artery and coronary artery rings. In contrast, compound C did not affect endothelium-dependent relaxations to acetylcholine and those to sodium nitroprusside. Moreover, RWPs induced the phosphorylation of AMPK at threonine 172 and eNOS at serine 1179 in endothelial cells within 10 minutes.
Conclusion: The present findings indicate that RWPs cause both NO and EDHF-mediated relaxations in several types of isolated arteries and that these effects are dependent on the activation of the AMP-activated protein kinase pathway.

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Ultrasound imaging of central artery and vein of the retina as a tool to evaluate perfusion of the retina
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Introduction: Spectral analysis of the Doppler signal recorded in the central artery and vein of the retina with colour-coded pulsed Doppler ultrasound imaging were compared in subjects suffering from central vein occlusion of the retina (CRVO) to normal subjects.
Methods: Twenty normal subjects (controls) aged from 25 to 50 years, and ten patients aged from 40 to 60 years (eight males and two female) suffering from CRVO were explored. Times between vein occlusion and the ultrasound study were ranged from 10 days to 720 months. Peak systolic (PSBFVca), end-diastolic (EDBFVca) and time–average mean (MeanBFVca) blood flow velocities were measured in the central artery at the level of the optic nerve few millimetres

upstream the retina using a colour pulsed Doppler imaging system with a 9 MHz linear device (Vivid 7, GE Medical Systems *ultrasound* ®, Horten, Norway). Maximum (MaxBFVcv), minimum (MinBFVcv) and time–average mean (MeanBFVcv) blood flow velocities were measured in the central vein downstream retina. Ultrasound imaging of carotid arteries was performed to rule out patients with carotid stenosis.
Results: In normal subjects, in the central artery, PSBFVca was 12.9 ± 3.4 $cm.s^{-1}$, EDBFVca 4.8 ± 1.6 $cm.s^{-1}$, MeanBFVca 4.1 ± 1.1 $cm.s^{-1}$, in the central vein, MaxBFVcv was 5.1 ± 1.1 $cm.s^{-1}$, MinBFVcv 3.2 ± 0.7 $cm.s^{-1}$, and MeanBFVcv 2.4 ± 0.5 $cm.s^{-1}$. For eyes suffering from CVOR, PSBFVca was 9.6 ± 2.0 $cm.s^{-1}$, EDBFVca was 3.1 ± 0.7 $cm.s^{-1}$, MeanBFVca was 3.1 ± 0.5 $cm.s^{-1}$, ($P < 0.001$ vs. values in normal subjects), MaxBFVcv was 3.9 ± 1.6 $cm.s^{-1}$, MinBFVcv was 2.4 ± 1.2 $cm.s^{-1}$, and MeanBFVcv was 1.8 ± 0.7 $cm.s^{-1}$, ($P < 0.01$ vs. values in normal subjects). There was no difference between values of blood flow velocities in contra lateral healthy eyes and values in normal subjects.
Conclusion: Measurements of blood flow velocities in the central vein and the central artery of the retina are feasible with a good accuracy (0.7 $cm.s^{-1}$) . For the eye suffering from CRVO, arterial and venous blood flow velocities were significantly decreased compared with those measured on the healthy eye in the same subject and those of normal subjects. Thus, CRVO involved, upstream, a recognizable decrease in arterial blood flow velocities and an increase in vascular resistances in the whole vascular supply of retina. This could be explained by the vascular anatomy of the retina (terminal type) in which arterial and venous blood flow rates must be equal. Decrease in blood flow velocities do not seem to depend from the time between the occlusion and the ultrasound study.

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Did ACE inhibition protect skeletal muscle metabolism from acute ischemia-reperfusion effect?
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Introduction: Angiotensin-converting enzyme (ACE) inhibitors are involved in protection against energetic metabolism defect in chronic heart failure. We tried to determine if chronic ACE inhibitor administration could protect the mitochondrial function of skeletal muscle from acute ischemia-reperfusion effect.
Materials and Methods: Twenty-six Wistar male rats (≈300 g) have been separated in two groups : the first one was the control group (CONT, $n = 13$) and the second one the experimental group (ACE, $n = 8$). In the experimental group every animal was treated during 28 consecutive days by 40 mg/kg/day of captopril. In each group every animal was submitted to 5 hours of ischemia by a rubber band tourniquet applied on the left root of the hindlimb followed by 5 minutes of reperfusion, the right hindlimb being used as witness. Finally both gastrocnemius muscles have been excised in order to evaluate mitochondrial respiration by *in-situ* saponin skinned fibres method. Maximal oxidative capacities of the skeletal muscle (V_{max}) and complexes I, II and IV of the mitochondrial respiratory chain were determined using glutamate-malate, succinate and TMPD-ascorbate as substrates.
Results: In the control group left limb V_{max} was significantly reduced by ischemia (4.5 ± 0.4 vs 7.4 ± 0.5 µmol O_2/min/g dry weight respectively $P = 0.002$) in comparison with the right limb. In the experimental group, left limb V_{max} was also significantly reduced by ischemia (5.1 ± 0.6 vs 7.8 ± 0.9 µmol O_2/min/g dry weight respectively $P = 0.006$). There is no significant difference between treated and control rats concerning left limb V_{max} ($P = 0.41$), showing that ACE inhibitors have no protective effect. Mitochondrial complexes activity is altered by 5 hours of ischemia-reperfusion (complexe I: −35%, $P = 0.002$; complexe I : −50%, $P = 0.0003$) in the same way in treated and non treated rats.
Conclusion: Mitochondrial respiratory chain I and II complexes of skeletal muscles are significantly altered by prolonged ischemia followed by a short reperfusion period. In acute ischemia, muscular metabolism is not protected by ACE inhibitor treatment, but it could be interesting to study the recovery of muscular oxydative function on a midterm and long term basis.

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Induction of angiogenic factors in skeletal muscle of rat by low-voltage electrical stimulation
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Introduction: Promotion of angiogenesis is vitally important after acute arterial occlusion. Vascular endothelial growth factor (VEGF) is the major enhancing factor of the vessel growth. According to some recent reports the VEGF production in skeletal muscle could be promoted by electrical stimulation (ES), but the mechanisms of this effect have not been fully elucidated. In hind limb ischemia model of rats, we studied whether LVES induced vascular endothelial growth factor (VEGF) production was due to hypoxia, inflammation or other angiogenic factors.
Methods: Eight male Sprague–Dawley rats (mean body weight 375 g) were studied using the Takeshitás model of hind limb ischemia. After bilateral occlusion of the femoral arteries, an active electrode was implanted into the right tibialis anterior (TA) muscle (the left one served as control). ES was started the day of electrode implantation and continued for seven consecutive days (stimulus width 0.3 ms, frequency 50 Hz, and strength 0.1 V). After the end of ES, the animals were decapitated and immunohistochemical analysis (ELISA) for VEGF, hepatocyte growth factor (HGF), fibroblast growth factor (FGF), interleukin 6 (IL-6) and hypoxia inducible factor (HIF-1α) was done using polyclonal antibodies.
Results: Immunostaining of rat TA muscles with polyclonal anti-VEGF antibody and polyclonal anti-HGF antibody revealed an increase of VEGF and HGF in the stimulated muscles compared with the controls (VEGF: 89.1 ± 17.2 ng/g vs. 65.1 ± 2.9 ng/g; $P < 0.05$, and HGF: 8.5 ± 2.0 ng/g vs. 5.8 ± 2.1 ng/g; $P < 0.05$). There were no differences in the levels of FGF, IL6 and HIF-1αfactors between stimulated and non-stimulated muscles.
Conclusion: VEGF and HGF might significantly contribute to the local angiogenesis by ES in the model of skeletal muscle ischemia. The ES-induced VEGF production in this model was not related to hypoxia or inflammation. Therefore, ES could be regarded as an effective alternative for the tissue salvage in vascular ischemia.
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A specific microacoustical and mechanical method applied to identify defects in muscle elasticity related to various dystrophies
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Introduction: Defects in the structure or expression of components of the muscle fiber sarcolemma underlie a number of genetic myopathies such as deficiencies of dystrophin (mdx mice) or dysferlin (sjl mice). In order to improve the identification of such muscle pathologies, a new approach using specific high frequency ultrasonic indentor (20–80 MHz) has been designed. It was tested on various muscles (diaphragm, pectoral, abdominal) to detect any differences.
Methods: Thanks to this specific indentor, it is possible to assess in one measurement the thickness, the acoustic properties (longitudinal velocity and attenuation) and the sample stiffness which can be related to an elastic modulus. Before the measurements, mdx, sjl and control tissues were dissected and rapidly conserved in Krebs solution.
Results: On one hand, we observed a large increase of the attenuation and thickness in dystrophin-deficient mdx mice. On the other hand, the results in sjl mice reveal behaviours like control mice. However, the sjl tissues thickness is much smaller than control tissues. These different results indicate a correlation between the muscle structure abnormalities and elastic properties. Indeed these elastical parameters show the difference between non-muscular area present in mdx mice (presence of fibrosis, fatty, macrophages, and necrotic fibers) and muscle area. However, the elastic behaviour of the diaphragm was evident and this property was clearly specifically correlated with each of the muscle pathologies studied. In fact, we assume that, unlike abdomen or pectoral where muscle fibers have rather large section, the small size of all diaphragm fibers may be responsible for the elastic properties of this muscle.
Conclusion: This work shows the feasibility of this new method in improving membrane elastic defects evaluation. The aim of this work is finally to use this method to compare biophysical properties after specific therapeutic treatments. Now, we plan to investigate whether the muscle degenerescence evolution, particularly severe in these pathologies, is also distinguishable by such micro-acoustic methods when applied to muscle from older animals.

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Peripheral venous distension and susceptibility to hyperventilation-induced central apnea in anaesthetized sheep
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Introduction: The mechanisms involved in the genesis of central sleep apnea in severe congestive heart failure (CHF) patients remain poorly understood. Despite profound circulatory changes in CHF and the fact that information originating from cardiovascular system can affect the control of breathing, the role of circulatory factors in the genesis of central sleep apnea in heart failure has never been really investigated. It is usually assumed that the main circulatory factor responsible for hyperventilation-induced central apnea results from left heart failure-induced lung congestion. Such a contention is however far from being proven. We have investigated the possible contribution of the load imposed on venous return (one of the main consequences of CHF) based on recent results suggesting that ventilation is stimulated during the congestion of the venous system in the skeletal muscles (1).
Methods: We studied in anaesthetized sheep the effects on the propensity for hyperventilation-induced apnea of obstructing the right ventricle and the inferior vena cava, using intravascular balloon catheter inflations and pressure support ventilation technique.
Results: Both types of obstruction were able to increase minute-ventilation (+0.5 ± 0.3 l/min) and decreased the value of arterial PCO_2 required to elicit an apnea (AT $PaCO_2$ = −5 ± 2 Torr). Compared with control, the difference between eupnea $PaCO_2$ and AT $PaCO_2$ decreased by −2 ± 1.2 Torr.
Conclusion: These results suggest that the load imposed on the venous return increases the propensity for hypocapnia-induced apneas, regardless the status of the pulmonary circulation and the left heart.

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Reduction in histamine-induced lung ventilation heterogeneity by PEEP: a synchrotron radiation computed tomography (SRCT) study in rabbit
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Introduction: Severe broncho constriction produces considerable heterogeneity in regional lung ventilation. The goal of this study was to measure the effects of PEEP on regional lung ventilation heterogeneity.
Methods: We used SRCT ventilation imaging in an anaesthetized, mechanically ventilated rabbit (1, 2). Maps of regional specific ventilation (sV) were obtained consecutively at PEEP = 0, PEEP = 5, and PEEP = 0 cm H_2O, at baseline and 16 min following histamine (125 mg/mL) aerosol administration. Ventilation heterogeneity was measured as the CV within the sV map.
Results: Following histamine, 5 cm H2O PEEP had a very small effect on mean sV, but strongly decreased ventilation heterogeneity. This effect partly remained 7 min following PEEP administration.
mV, minute ventilation.
Conclusion: A PEEP of 5 cm H_2O reduced regional ventilation heterogeneity due to histamine-induced broncho constriction, without a prominent effect on sV. Further experiments are underway to confirm this effect and to elucidate its mechanisms.

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Increased uncoupled mitochondrial oxygen consumption in glycolytic skeletal muscle from mice expressing human uncoupling protein 3
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Introduction: Excessive whole body energy storage could lead to obesity and its related co-morbidities such as type II diabetes. Methods performed to increase metabolic rate would then contribute to reduce fat mass development, limiting the prevalence of obesity and its related disorders. Mitochondrial oxygen consumption in skeletal muscle contributes to the whole body energy expenditure through fatty acid and NADH/FADH2 oxidation. This process corresponds to a transfer of electrons from substrates to oxygen which moves protons out of the mitochondrial matrix; this proton leak generates a chemiosmotic gradient ensuring ATP synthesis and uncoupled respiration by a futile cycle caused by uncoupling proteins. The objective of the present study was to determine if increased uncoupling protein 3 (UCP3) expressions can promote mitochondrial oxygen consumption in mice glycolytic and oxidative skeletal muscles.
Methods: Transgenic human UCP3 mice were created on a B6D2 genetic background. The mRNA overexpression of UCP3 was from 5 to 8-fold depending on the muscle analysed. At 12 weeks of age, animals were killed and tibialis anterior and soleus muscles were immediately removed for respiratory measurements. Mitochondrial oxygen consumption was measured at 25°C on saponin-permeabilized muscle fibers from six transgenic and six wild-type animals from the same littermate with a Clark-type electrode (Hansatech Instruments, UK). Respiratory rates were recorded after successive additions of Glutamate/Malate (5/2 mM), ADP (500 µM), oligomycin (10 µg/mL) and FCCP (1 µM).
Results: UCP3 overexpression increased basal state four oxygen consumption (1.33 vs. 0.49 nmol oxygen/min/mg dry tissue for transgenic and control mice respectively) and reduced significantly oxidative phosphorylation efficiency as determined by the ratio ADP stimulated / basal oxygen consumption (3.2 vs. 8.1) in tibialis anterior muscle. Chemical uncoupling of mitochondrial respiratory chain neutralized differences between transgenic and wild type animals, showing the specificity of UCP3 action. In agreement with the weak human UCP3 expression in oxidative muscles, no effect was observed in the soleus muscle. Increased basal mitochondrial related skeletal muscle energy dissipation could explain the lesser increase in body weight observed in transgenic mice fed on a high fat diet.
Conclusion: These data suggest that manipulating respiratory uncoupling in muscle could represent a promising treatment for obesity and its metabolic disorders.

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A study approach of the maximum expiratory flow-volume curves
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Introduction: The object of this work is to develop a computer processing system allowing an analysis of the morphology of the maximum expiratory volume flow-curve (MEVF) leaving out the volume. This tool was developed in C++ et Visual Basic for Jeager Masterlab (Jaeger Viasys Healthcare, Hochberg, Germany) and Pneumo-multitest (Erems, Flourens, France). Currently, the results that have been returned during a MEVF correspond to an analysis of the expiratory flow in value of particular points of the graph obtained (maximum expiratory flow or MEF and MEF when 75%, 50% and 25% of the forced expiratory vital capacity). The data-processing tool that we propose makes it possible to record the patient's information (age, sex...) and to carry out a MEVF-model by derivative analysis. This model makes it possible to appreciate on the one hand the speed of the beginning of the expiratory phase and thus the participation of the subject during the investigation, and on the other hand, to appreciate the deceleration of the expiratory flow and thus to evaluate degree and localization of the bronchial obstruction.
Methods: Unlike to the calculation of tangents proposed by *J. Mead* until 1978, this is a model based on 'the closest slopes'. At the beginning, a curve is modelized by a segment going from the maximum to the end of the exhalation. If the model strays away too much from the real curve (above a chosen threshold), the maximum difference point is processed thus dividing the initial segment into two distinct slopes. The procedure continues determining possible new segments.
Results: A test folder was created based on the results about subjects representing the three principal ventilatory syndromes. The three great syndromes of the ventilatory function were successfully distinguished thanks to the determination of the number and the value of slopes determined in the expiratory phase.
For example: a healthy patient shows a single slope of –2.3. A patient presenting an obstructive ventilatory insufficiency syndrome characterized by a reduction in the MEF 25 shows two slopes, one of –5.95, the other of –2.00. The combination of the slope values and the localization of the point of intersection of these ones, being after a third of the expired volume, must make it possible to appreciate the level of the obstruction. In this case, it can be located on the level of the small airway.
Conclusion: In order to validate these last assumptions, new measurements are needed and developments are planed, in particular in order to create a user interface for this tool that would make it compatible with portable systems of exploration of ventilatory flow. This expiratory flow analysis tool has to detect early obstructive ventilatory syndromes while remaining independent from the expired volume and has to evaluate their evolution.

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Exploration of the upper airway collapsibility by negative expiratory pressure
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Introduction: Polysomnography in a sleep laboratory is the widely accepted standard method used to diagnose obstructive sleep apnoea syndrome (OSAS).The procedure is, however, expensive and time consuming. In such condition, simpler and less expensive tests are needed. So the objective of this study was to assess parameters of the NEP technique, in both seated and supine positions, other than expiratory flow limitation (EFL) able to detect OSAS in snoring patients.
Material and Methods: It is a transverse study concerning 42 OSAS diagnosed by PSG, 34 simple snorers and 32 healthy subjects. Lung function was measured by using a plethysmograph and a NEP technique performed in the seated and supine positions in a random order. The depression was fixed to 5 cmH_2O.
Results: All patients of the study had normal forced expiratory flow/volume loops. Apnoeic patients had lower Dflow in both positions with a number of the oscillations on the expiratory curve obtained with NEP and an EFL in supine position more raised than other groups ($p < 0.05$). Passage from the sitting to the supine position raised LDE of the three groups with a significant fall of Dflow and an increase of the number of oscillations in snoring and OSAS patients ($p < 0.05$). A significant correlation was found between apnoea-hypopnoea index (IAH) and LDE and between Dflow and IAH especially in supine position ($p < 0.05$). The analysis of variance showed that difference was significant between the 3 groups of the study concerning LDE and number of oscillations with respective sensitivity of 83% and 90%. Post - hoc comparison showed that only the number of oscillations was significantly different between apnoeic and snoring patients.
Conclusion: NEP constitutes a simple and useful tool for the screening OSAS. LDE and especially number of oscillations on the expiratory curve obtained with NEP constitute the most sensitive parameters for the screening of this syndrome.

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Effects of birth morphology, sex, socioeconomic status and environmental factors on the development of the lung function in healthy Tunisian children
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Introduction: The aim of this study was to determine the relationship between lung function and the anthropometric, socioeconomic and environmental factors on lung function in healthy Tunisian children.
Methods: The specific parameters of pulmonary function included forced vital capacity (FVC), forced expiratory volume in one second (FEV_1), peak expiratory flow (PEF), maximal expiratory flow at 50% of FVC (MEF_{50}) and maximum mid expiratory flow between 25% and 75% ($MMEF_{25–75}$) were measured with a portable spirometer (Minato) in 764 asymptomatic, non-smoking, healthy Tunisian children 6 to 16 years of age (390 boys and 374 girls, mean age: 10.6 ± ?0.1 years). Morphology at birth, socioeconomic status, indoor and outdoor pollution were evaluated by an auto-questionnaire. The statistical analysis was performed using SPSS. Data were expressed as means ± sem. We compared the means with the Kruskal–Wallis test. We transformed each functional respiratory test into a binary variable y (0.1) separating high values from low values according to their relation with age, the cut-off point being obtained by a regression tree. A step by step logistic regression was performed to explain functional respiratory tests by significant and independent risk factors.
Results: The adjusted risk to have a low pulmonary function was divided by around 2 by additional year: OR ≃ 0.4 (0.35; 0.45) $P < 0.000\ 0001$; the same adjusted risk attached to height was ≃0.85 (0.84; 0.88) $P < 0.000\ 0001$, by additional centimeter. The major and protective effect of the age (or the height) ruled out, the adjusted expression of aggravating risks showed that predictive factors linked to low pulmonary functions were: female sex for FVC and PEF (OR 2.00 and 1.67 and $P < 0.001$ and 0.01 respectively), low birth height for FVC (OR 1.1/fewer centimeter, $P < 0.02$), civil servant or workers vs. owners, directors or doctors for FEV1 (OR 2.90 and 4.20, $P < 0.005$ and 0.001 respectively), and gas heating vs. electricity and outdoor pollution for MMEF 25–75 (OR 1.92 and 2.40, $P < 0.008$ and 0.02 respectively).
Conclusion: The main factors affecting the development of pulmonary function during childhood were the morphological factors (age and height). However, if these factors were ruled out, sex, birth height, socioeconomic status and environmental conditions may influence the lung development, in a separated way according to the functional criteria. This heterogeneity could be explained by the fact that each measured values describe a different structure.

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Effect of active sensitisation to *Dermatophagoides pteronyssinus* allergen on airway function in brown Norway rats
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Introduction: The aim of this study was to develop and characterize a model of asthmatic disease in brown Norway rats sensitized to *Dermatophagoides pteronyssinus* allergen (D pter).
Methods: Male brown Norway rats (250–300 g) ($n = 7$) were sensitized by 2 subcutaneous injections of D pter (50 IR/ml) (Stallergenes AS, France) and Al_2O_3 at days 0 (D0) and 3 (D3), followed at D17 by intratracheal instillation of D pter. Control (C) rats ($n = 5$) underwent the same protocol but with saline solution instead of D pter. Witness (W) rats ($n = 5$) were not submitted to any treatment. At D24, enhanced expiratory pause (Penh), used as an index of airway resistance, was measured using a barometric plethysmograph for conscious animals (Buxco, Troy, NY). At D25, a bronchoalveolar lavage (BAL) was performed, and isometric contraction was measured on rings isolated from trachea (T), extrapulmonary (EPB) and intrapulmonary bronchi (IPB) using an organ bath system (EMKA, France). Maximal contraction (F_{max}) and EC_{50} were derived from cumulative

concentration response curves to carbachol (CCh) (10^{-8}–10^{-3} M). Results are expressed as mean ± SEM. Statistical comparisons were done by non parametric tests using SPSS software. Differences were considered significant when $P < 0.05$.
Results: In response to allergen challenge, Penh was increased in S, but not in C and W rats. *In vitro* stimulation by D pter also specifically induced contraction of T, EPB and IPB rings isolated from S, but not C and W rats. In response to metacholine (MCh) challenge (10^{-8}-3×10^{-1} M), MCh concentration inducing 300% increase in Penh was significantly lower in S (–4.36 ± 0.44) and C (–3.92 ± 0.54) versus W (–2.86 ± 0.55) rats. F_{max} to CCh was significantly higher in IPB from S (1966 ± 232 mg/mg wet weight) versus C (1613 ± 189 mg/mg wet weight) and W rats (1191 ± 113 mg/mg wet weight), and EC_{50} significantly lower (S: –6.22 ± 0.03; C: –5.64 ± 0.02; W: –5.23 ± 0.02). In BAL fluid, cellular density was significantly higher in S (334 ± 52 cells/μL) versus C (217 ± 18 cells/μL) and versus W (165 ± 52 cells/μL) rats, as well as the percentage of eosinophils (S: 8.75%; C: 1.74%; W: 0.7%) and mast cells (S: 0.5%; C: 0.2%; W: 0%).
Conclusion: Brown Norway rats sensitized to D pter showed (i) *in vivo* and *ex vivo* specific bronchoconstriction to allergen stimulation, (ii) *in vivo* hyperresponsiveness and *ex vivo* hyperreactivity and hypersensitivity to cholinergic stimulation, and (iii) increased proportion of eosinophils and mast cells in BAL fluid, indicating that such sensitized rats are a relevant model of asthma.

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Simplified assessment of insulin sensitivity (SI) over 2h after a standardized hyperglucidic breakfast (SHB)
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Introduction: Insulin sensitivity (SI) is an important physiological parameter but remains complex and expensive to measure accurately. However beside the reference methods [glucose clamp, minimal model analysis of an intravenous glucose tolerance test (IVGTT)] recent studies have emphasized the accuracy of a simpler approach: the minimal model analysis of a standardized hyperglucidic breakfast (SHB), i.e., a "physiological" procedure to assess glucose tolerance, that is also suitable for the diagnosis of reactive hypoglycemia. We previously validated the measurement of SI with the analysis of the three postload hours of a SHB. We investigated here whether the same analysis performed over only 2 hr is a reliable and even simpler alternative to the full sampling procedure over 3 hr.
Methods: A total of 22 subjects underwent a SHB (495 kcal, 76 G of carbohydrates) analyzed over 3 hr. Caumo's "Oral minimal model" (OMM) was applied on SBT values with two procedures: classical calculation over 3 h (9 samples for blood glucose and 9 for insulin) as previously validated, and a calculation performed only over the initial postload 120 minutes (only five samples for blood glucose and five samples for blood glucose). We compared values of SI given by the 3 hr and the 2 hr procedure.
Results: Values of SI ranged from 1.9 $min^{-1}/(\mu U/mL\ 10^{-4})$ (insulin resistance) to 35 $min^{-1}/(\mu U/mL\ 10^{-4})$ (high insulin sensitivity). The analysis shows an almost total agreement between the determinations on 3 h and 2 h ($y = 1.3617x–1.8737$; $r = 0.984$; average deviation 0.51, confidence interval -0.65 to 1.68) with only one over-estimate for a very high value of SI which is traditionally less reproducible with the minimal model whatever the technique employed. There is no misclassification of any individual in the spectrum of insulin sensitivity values.
Conclusion: Therefore, 2 hr are enough to assess SI with a SHB if one uses Caumo's OMM procedure. The use of five rather than 18 (as in IVGTT) paired values of insulin and glucose yields a 72% reduction in reagents-related cost. Such procedures, both simple and robust, may allow a more usual determination of SI in clinical practice instead of simple popular indices based on fasting insulin and glucose levels which are actually unreliable in most clinical situations.

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Heterogeneity of GH effects on aerobic power capacity and metabolism in adult GH-deficient patients
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Introduction: GH treatment in adults is known to improve fitness outcomes. We investigated whether the amplitude of this effect is related to the other effects of GH on fuel metabolism and body composition.
Methods: A total of 18 GH-deficient adult patients underwent an exercise calorimetry to assess the balance of substrate oxidation at exercise at the onset, at the 12th month and at the 24th month of GH treatment.
Results: Twelve patients markedly increased [more than 20% their maximal power output (Pmax)] whereas in the six others there is very little or no increase in Pmax (<20%). The two groups are matched for age, BMI, body composition, blood glucose and blood lipid profile. In those whose Pmax increases, fat-free mass increases from 45.37 ± 2.76 to 47.08 ± 2.80 kg ($P < 0.05$) and the ability to oxidize lipids at exercise is improved with a maximal value (LIPOXmax) occurring at a higher power intensity (before: 36.17 ± 5.4 after: 48.76 ± 5.35 Watt $P < 0.05$) with a flow rate of lipid oxidation rising from 72.18 ± 13.9 to 143.42 ± 12.65 mg/min ($P < 0.05$). After 2 years fat-free mass continues to increase up to 50.7 ± 3.20 kg ($P < 0.02$), as well as the maximum flow-rate of the lipid oxidation (that reaches 161.8 ± 22.8 mg/min $P < 0.05$) while a decrease in LDL-cholesterol becomes significant from 1.29 ± 0.12 to 1.06 ± 0.14 g/L ($P < 0.05$), correlated with a decrease in total cholesterol (from 2.12 ± 0.13 to 1.78 ± 0.18 g/L $P < 0.05$). By contrast patients whose Pmax did not increase exhibit at 12 months an increase in fasting blood glucose (from 4.42 ± 0.22 to 5.08 ± 0.18 mmol/L; $P < 0.02$) proportional to an impairment in the balance of substrate oxidation at exercise evidenced by a stronger reliance on CHO whose rate of oxidation rises from 0.28 ± 0.02 to 0.33 ± 0.03 mg/min/kg/watt $P < 0.05$).
Conclusion: Therefore the beneficial effects of GH on metabolism and body compostion occur mostly in patients whose ability to exercise improves under treatment, while in those whose fitness is not improved CHO metabolism is rather deteriorated.

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Biologic profile in tunisian infants hospitalized for malnutrition
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Introduction: Malnutrition is one of the principal causes of morbidity and mortality in the old children of less than 5 years. In Tunisia, the malnutrition remains again a problem preoccupying in public health and notably when it touches young children less than five years. Malnutrition affects various significant functions such the physical growth, the mental development, the capacity of training and the immnunizing response to the infections. It was reported that a deficit even medium of zinc, selenium, iron, copper, vitamins A, C, E and B6. The available data in the literature, concerning notably the effect of the hypothyroïdie on the profile lipidique and liporotéinique, and its relation with the athérosclérose comes from study achieved at adults. In Tunisia, the struggle against the micronutriments deficiencies has always constituted a priority of the Ministry of the Health.
Methods: To situate the gravity of these deficiencies and their impact on the health in children, we have realized a study including two groups of infant less than 2 years old: a control group and a malnourished group.
Results: Our data consolidate the important impact of pregnant women nutritional state and of breastfeeding on the fetus and infant growth. Compared to the control group, the malnourished young showed a significant alteration in the levels of several biological parameters. The malnourished infants showed, notably, a significant reduction of the average values of Chol-HDL, apo-A1, VitE, TSH and FT4 levels and Chol-HDL/Chol-LDL ratio.
These data showed the deep biologic disruption in the malnourished children and the importance that the nutritional state of the pregnant woman and the maternal nursing can have in the growth of the fœtus and the baby. In addition, Chol-HDL, apo-A1 and Chol-HDL/Chol-LDL were found positively and significatively correlated with FT4. So, these reduction in ill infants seems to be linked with a reduction of the thyroid function.
Conclusion: Our results confirm the existence of an important change of biological profile in malnourished young infants. In addition, the study shows the necessity to target and to develop means of struggle against factors favoring the malnutrition. One thinks that in our future studies, it would be useful to examine, at youngsters (hypothyroïdiens, dénutris and normal), the variation of the rate of the thyroid hormones and the quantitative evolution of the lipoprotéines sériques.

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Thyroid functional structure and body weight studied in the streptozotocin and insulin treatments
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Introduction: The thyroid is a master endocrine gland that plays a fundamental role in the intermediary metabolism of all tissues. The effects of the thyroid result from the biosynthesis and secretion of two hormones, triiodothyronine (T3) and thyroxine (T4). The regulation of this thyroidienne function is under control by the hypothalamo-hypophysial axis and others hormones intervene too like insulin.
Methods: The streptozotocin and insulin are used to induce hypo and hyperinsulinemia in the rat Wistar model. We measure the effects of these treatments on the functional structure of thyroid follicular, the body weight and the take of food and water. Four groups are constituted; each one is formed of two batches: one is treaty, the second is control. Solution of NaCl 0.9% was injected to all pilots' batches. The treated batches of the first and of the second groups were treated by streptozotocin (STZ) 40 mg/kgBW. The first is sacrificed thirty five days later and the other forty five days. The third group is treated by dose of 65 mg/1KgBW of streptozotocin during thirty days and the last group is daily treated by the insulin at the dose of 200 μUI/gBW.
Results: We note that there are important structural change in the thyroid gland, the comparison of the batches treated by the streptozotocin and insulin with the witness batches we noted that the diameter colloid batch insulin is smaller than the witness which has a diameter smaller than those treated by the STZ and conversely for the length of the epithelium, that are accompanied by marked alteration in their secretory activity that hormonal and biochemical proportioning will confirm. There are changes in body weight and take of food and water witch are statistically significant difference, calculated by test student and ANOVA, we observed the variation of the evolution of the body weight between these different groups.
Conclusion: All this changes are bound of to variation of insulin concentration. The action of insulin on the gland thyroid is exerted on the activation of some enzymes of the synthesis of the thyroid hormone like TPO, TG or TTF. When the insulin rate decreases the thyroid activity is inhibited so a colloid abundant and conversely in the hyper insulinemia the thyroid activity is increased thus a small quantity of colloid. The variations of the concentration of insulin in the circulation generate variation of the effects depending on it. From these experiments we contributed has to show that the sphere of activities of the insulin extended to the thyroid gland where it exerts an influence on the various metabolic activities which depend on this gland.

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Membrane cholesterol modulates Kv1.5 subunit distribution and Kv1.5-based channel function in rat cardiomyocytes
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Introduction: Lipids are major determinants of channel function. They greatly influence the structural and physical properties of the plasma membrane. Two major lipids of the plasma membrane, cholesterol and sphingolipids, pack tightly together to form microdomains called 'lipid rafts'. Lipid rafts are dynamic platforms important for the delivery of proteins to the membrane and for sequestering proteins in close physical proximity to control their functional interactions. An increasing number of channels has been found to be targeted into these cholesterol and

sphingolipid rich membrane microdomains, including Kv channels. In this study, we assessed the role of membrane cholesterol composition in the distribution and function of Kv1.5-based channels in rat cardiac membranes.
Methods: Atrial and neonatal cardiomyocytes were enzymatically isolated, and elecrophysiological studies were done with patch clamp technique, in whole cell configuration. Neonatal cardiomyocytes maintained in culture were transfected with 0.5 µg of GFP-tagged Kv1.5 expression vector using FuGENE® 6 Tansfection Reagent. We used the sucrose gradient technique, with immunoblot analysis, to define the microdomain in which Kv1.5 channels were localized. Confocal microscopy was used to visualize GFP-tagged channels expressed in living cells and immunostainings performed in myocardial sections.
Results: In isolated rat atrial myocytes, the application of 2% methyl-β-cyclodextrin (MCD), an agent that depletes membrane cholesterol, caused a delayed increase in the Kv1.5-based sustained component, I_{sus}, which reached steady-state in ~7 minutes. This effect was prevented by preloading the MCD with cholesterol. MCD-increased current was abolished by 500 µM 4-AP. Neonatal rat cardiomyocytes transfected with GFP-tagged Kv1.5 channels showed a large ultrarapid delayed-rectifier current (I_{Kur}), which was also stimulated by MCD. In atrial cryosections, Kv1.5 channels were located at the intercalated disk, whereas caveolin-3 predominated at the cell periphery. A small portion of Kv1.5 floated in the low-density fractions of step sucrose-gradient preparations. In live neonatal cardiomyocytes, GFP-tagged Kv1.5 channels were predominantly organized in clusters at the surface membrane. MCD caused reorganization of Kv1.5-subunits into larger domains, an effect that was sizable 7 minutes after drug application.
Conclusion: We conclude that Kv1.5-subunits are concentrated in cholesterol-enriched membrane microdomains distinct from caveolae, and that redistribution of Kv1.5-subunits by depletion of membrane cholesterol increases their current-carrying capacity.

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Expression of TRPC3 / 6 in human jurkat T cells
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Introduction: In this study, we investigated the presence of mRNA, encoding for two members of transient receptor potential (trp) calcium channel family, i.e., TRPC3 and TRPC6, in human jurkat T cells. These channels are activated by fatty acids and, particularly, by diacylglycerols (DAG).
Methods: In this study, we employed RT-PCR technology in order to identify TRPC3 / 6 channels in human jurkat T cells before and after stimulation with different mitogene like phorbol 12-myristate 13-acetate (PMA) plus Ionomycin and anti-CD3 antibodies which exert different mechanisms of action in the activation of T cell proliferation. The jurkat T cells are rendered quiescent by overnight incubation in the absence of serum and then stimulated by these mitogens.
Results: We correlated TRPC expression with the transcription of IL-2 mRNA in these cells. We observed that there existed a corelationship between the expression of TRPC3 / 6 mRNA and IL-2 mRNA. TRPC6 is expressed in the early phase of T cell activation whereas TRPC3 expression is related to the later phase of cell proliferation induced by either PMA plus Ionomycin or anti-CD3 antibodies. IL-2 mRNA was expresed at 2 hours of stimulation by afore-said agents. The expression of TRPC mRNA was quickly induced (15 min) during T cell stimulation, followed by the expression of TRPC6 and, later on, IL-2 mRNA. Currently, we are investigating the activation of these channels by different dietary fatty acids and DAG-containing these agents.
Conclusion: These results show an association between TRPC3 / 6 mRNA expression and the different stages of cell cycle progression in human jurkat T cells. At present, we are conducting experiments on the inhibition of these channels by employing siRNA technology. We will present our results on the activation of these two calcium channels in the light of afore-mentioned observations.

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Expression of mRNA for RhoA-guanine exchange factors is modulated by angiotensin type I receptor in rat aorta smooth mucle cells
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Introduction: Angiotensin II (ANGII) has been implicated in various cardiovascular diseases. Therefore, characterization of the ANGII type I receptor (AT1R) signaling pathway, which is dominantly expressed in vascular smooth muscle cells (VSMCs), is essential to understand ANGII pathophysiological responses. Some of the ANGII cellular effects are mediated through the RhoA/ Rho-kinase signalling pathway, known to be involved in many aspects of cell functions like motility and transcription. The activation of RhoA by external stimuli is directly dependent of guanine exchange factors (GEFs). The aim of this study is to characterize the regulation of RhoA-GEFs mRNA expression by AT1R in VSMCs.
Methods: Rat RhoA activating-GEFs (29) have been selected and specific primers have been designed and validated for real time PCR. Aorta smooth muscle cells (ASMCs) from Wistar-Kyoto rats were cultured in DMEM with 10% calf serum and deprived in serum 24 h. GEFs mRNA expression levels were analysed in cultured ASMCs incubated with 1 µM of AT2R antagonist PD12319, with or without 100 nM ANGII for 1, 6, 12 or 48 h in the presence or absence of losartan (1 µM), an AT1R antagonist, and fasudil (1 µM), an inhibitor of Rho-kinase.
Results: RhoA-GEF mRNA expression profile analysis revealed three groups of distinctively expressed GEFs in non-stimulated ASMCs. The first group contained six GEFs which were predominantly expressed in cultured ASMCs. The second group was composed of thirteen GEFs with a moderate expression in ASMC and a third group which represented the nine RhoA-GEFs showing a low expression level. Obscurin was not detected in cultured ASMCs. Transcriptional modulation of RhoA-GEFs by ANGII-stimulated ATIR was observed at 1, 6, 12 and 48 hours of stimulation. Analysis of GEFs mRNA expression revealed transient variations of few RhoA-GEFs (scambio, p190, CDEP, $n = 5$), an early (1 h) and maintained down-regulation of the Rhogef tech ($n = 5$), and a late (48 h) down-regulation of a group of 9 RhoA-GEFs mRNA including arhgef 1, 2, 11, 12, 18, BCR, ECT2, p63Rhogef and vav2. This ANGII-induced rhoA-GEF down-regulation was reversed by losartan ($n = 5$). As Rho-kinase plays important role in ANGII-induced modulation of mRNA expression, fasudil was tested and we observed a loss of GEF-mRNA down-regulation except for p63RhoGEF and Arhgef 11 ($n = 5$).
Conclusion: These results describe the RhoA-GEF expression profile in ASMC and show that RhoA-GEFs are transcriptionaly modulated by AT1R-AngII stimulation through RhoA/Rho-kinase dependent and independent pathways. These observations suggest the presence of complex feedback mechanisms between ANGII-induced RhoA activation and the transcriptional regulation of its activators.

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Effect of specific respiratory muscle training in mdx mouse on calcium homeostasis and mitochondrial respiration
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Introduction: The cellular mechanisms resulting from the low exercice capacity of respiratory muscles in children with Duchenne muscular dystrophy (DMD) are poorly known. The aim of this study was thus to investigate the functional effect induced by a specific respiratory muscle training in *mdx* mouse diaphragm.
Methods: Respiratory muscle training, was performed by hypercapnic stimulation of ventilation (8% CO_2), during 6 weeks/30 min per day, in 10 *mdx* mice (10 *mdx* mice were also used as control).
Results: Diaphragmatic force frequency relationships showed that this specific respiratory training improves the tetanic force production of the diaphragm. Maximal mitochondrial respiration was increased in the training group (14.3 ± 0.4 vs. 9.5 ± 0.3 µmol/mn/mg) with no difference between the groups in diaphragm muscular fiber type and in citrate synthase activity. In additon, ryanodine receptor (RyR) activity was extrapolated from the analysis of spontaneous Ca^{2+}-sparks measured on individual diaphragm fibers using laser scanning confocal microscopy. It showed decrease of Ca^{2+}-spark rise-time in training group (4.66 ± 0.11 vs. 5.59 ± 0.12 ms) without any difference in Ca^{2+}-spark amplitude, which could be interpreted as a better synchronisation of RyR opening involved in spark generation.
Conclusion: In conclusion, functional beneficial effect of low level of exercise on mdx diaphragm could be partially explained by an improvement of mitochondrial function and RyR gating properties which could attenuate the basal rise of cytoplasmic Ca^{2+}.

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Regulation of TRPC-dependent calcium influx by dystrophin/α-syntrophin complex: implication in Duchenne muscular dystrophy
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Introduction: Duchenne muscular dystrophy is a neuromuscular disease which leads to a progressive degenerescence of muscles. This disease results from the lack of dystrophin, a protein located at the cytoplasmic face of the sarcolemma. In normal skeletal muscle cells, dystrophin is associated with actin and complex called DAP (Dystrophin Associated Protein) linking actin cytoskeleton and extracellular matrix. The lack of dystrophin leads to fiber necrosis and is proposed to be mediated by calcium influx deregulation leading to an increase in the free calcium concentration under the sarcolemma. The source of this abnormal calcium influx phenomenon is SOC (Store Operated Channel) carried by TRPC1/TRPC4.
Methods: The aim of our study is to identify the proteinic environment of SOC by co-immunoprecipitation technique and to know the mechanism of SOC regulation by quenching of fura-2/AM by manganese and by patch-clamp. This, can give us news therapeutics targets (pharmacology therapy for example).
Results: We demonstrated that TRPC1 and TRPC4 form a stable complex with dystrophin and α-syntrophin through its PDZ domain. This last protein, which is a scaffolding protein, could mediate a molecular link between the dystrophin based cytoskeleton and TRPC1/TRPC4. But, less TRPC1 was co-immunoprecipitated with α-syntrophin from *mdx* muscle than from normal muscle. The decrease of this complex is likely due to the reduction of α-syntrophin at the sarcolemma in dystrophic muscle cells. This reduction could explain the enhancement of SOCEs (Store Operated Calcium Entries) by overactivation of SOC. To explore the functional regulation of SOCEs by α-syntrophin, experiments were designed to overexpress α-syntrophin in cultured myotubes. The measurement of calcium influx by quenching of fura-2/AM by manganese showed that forced expression of α-syntrophin reduced SOCEs which are abnormally elevated in dystrophic cells.
Conclusion: These results are in favour of the idea that the link of TRPC1 with dystrophin
and α-syntrophin could be essential for maintaining a normal regulation of SOC activity. Overexpression of α-syntrophin could be a therapeutic approach that needs to be considered. Pharmacologic step in order to specifically inhibit TRPC-dependent SOC could show a direct correlation between this inhibition and regression of cellular death observed in the dystrophic phenotype.

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Adaptation of vascular myocytes to microgravity by the decrease of ryanodine receptor subtype 1 expression
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Introduction: In space, the decrease of gravity modify blood spreading and induces the adaptation of the cardiovascular system. The main effect of microgravity on vascular function is the decrease of vascular tone leading to failure of orthostatic blood pressure after the spationaut return. Vascular contractility depends on calcium signalling. Both RYR1 and RYR2 ryanodine receptor subtypes are implicated in calcium signalling triggered by depolarisation or vasoconstrictor agents such as angiotensin II. In the hindlimb suspended rat (animal model used for microgravity studies), we have shown that a decrease of ryanodine receptor expression was responsible for the lost of vascular tone. To know if the decrease of ryanodine receptor expression could be observed in cells

cultured in microgravity, we used vascular myocytes cultured in specific boxes (MAMBA) placed in incubator (Kubik) and boarded in taxi-flight to International Space Station.
Methods: The incubator has two compartments, one exposed to microgravity and the other is on a centrifuge that reproduce gravity allowing the comparison of two populations of myocytes bearing the same culture and travel conditions but submitted or not to microgravity. Cells were fixed either at the beginning (flight effects) or the end of the flight (adaptation to microgravity) and expression of ryanodine receptor subtypes by RT-PCR and immunostaining was measured after Earth return.
Results: Our study shows that only RYR1 and RYR3 ryanodine receptor subtypes were expressed in cultured cells. The expression of RYR1 and RYR3 subtypes observed in centrifuged cells was similar to those observed in cells maintained in laboratory. A long exposure to microgravity decreased only the expression of RYR1 subtype whereas the flight did not modify the expression of RYR1 subtype. In conclusion, we suggest that vascular myocytes can adapt their contractility to microgravity by a decrease of RYR1 ryanodine receptor subtype that modify the calcium signalling activated by vasocontrictor agents.
Conclusion: This work is the first evidence that microgravity during flight induces molecular adaptation of calcium signalling in vascular myocytes.

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Evidence for functional coupling between mouse cardiac fibroblasts in primary culture
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Introduction: *In vitro* studies have shown that cardiac fibroblasts are able to establish heterogeneous interactions able to synchronize electrical activity with surrounding cardiomyocytes. The aim of our study was to identify the connexins involved in gap junction formation and to characterize their functional coupling.
Methods: Characterization of connexins was performed on cultured fibroblasts isolated from adult mouse heart by RT-PCR and Western blotting. Functional intercellular communication was measured by Gap Fluorescence Recovery After Photobleaching (gap-FRAP) method with carboxy-fluorescein as dye. To be able to observe fibroblast differentiation into myofibroblast, αSMA-RFP mice which expressed red fluorescence protein under the control of the promoter of alpha smooth muscle actin, a protein specifically expressed in differentiated myofibroblast, were used.
Results: Results show that mRNA and proteins of connexins 40 and 43 are both expressed in 12-day primary cultures of mouse cardiac fibroblasts. In addition, both the non-phosphorylated and phosphorylated forms of connexin 43 are present, suggesting that this protein could establish functional coupling. Functional analysis of cell to cell communication by the technique of gap-FRAP confirms that about 10% of fibroblasts are coupled. Their level of communication is increased in co-culture with cardiomyocytes (with the time constant of permeability $k = 6.6\ 10^{-2} \pm 0.5\ 10^{-2}\ min^{-1}$ in fibroblast culture [$n = 65$] increased to $k = 14.6\ 10^{-2} \pm 2.8\ 10^{-2}\ min^{-1}$ in co-culture of fibroblasts with cardiomyocytes, [$n = 108$]) but without a significant change in the number of communicating cells (10.8% versus 10.2%, respectively). As primary cultures of fibroblasts isolated from heart of αSMA-RFPmouse show that fibroblasts deeply differentiate into myofibroblasts during culture, this differentiation process could be of first importance in the establishment of cell coupling, which remains to be investigated.
Conclusion: These data show that cardiac fibroblasts expressed connexins 40 and 43 which were able to establish functional communications between cells and to form a coupled network. The amplitude and efficiency of this coupling could be function of the differentiation state of fibroblasts into myofibroblasts, which will be of particular interest in the perspective of cardiac cellular therapy.

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Myopathies with dystrophin deficiency: evidence for the involvement of IP3 calcium channels in the calcium deregulation and effect of IP3 pathway blockers
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Introduction: Skeletal muscle depolarisation induces a massive release of calcium stored in the sarcoplasmic reticulum (SR) through ryanodine receptors (RyR). Furthermore, previous data suggests that an elevation in myoplasmic 1,4,5-trisphosphate (IP3) may be a secondary triggering signal for SR calcium release. Evidence is presented here for the enhancement of an IP3-mediated calcium signaling pathway in myotubes from dystrophin-deficient cell lines as compared to mini-dystrophin transfected ones.
Methods: In this work, we performed experiments on two types of myotubes originating from the same Sol8 cell line: (1) dystrophin-deficient myotubes, SolC1 (−), and (2) myotubes transfected to express the BMD mini-dystrophin, SolD (+). Both Ca^{2+} releases after stimulation and Ca^{2+} sparks were investigated in these cell lines. To know if attenuation of such calcium releases could restore a non-pathologic context, cell survival assays (MTT test) were also performed in IP3 pathway attenuated conditions.
Results: Calcium rise induced by the perifusion of a solution containing a high potassium concentration, was higher in SolC1 (−) than in SolD (+) myotubes. At rest, the number of sites discharging calcium (release site density: RSD) was quantified and found more elevated in SolC1 (−) than in SolD (+) myotubes. The exposure to sarcoplasmic reticulum (SR) Ca^{2+}-channels inhibitors (ryanodine and 2-APB) and phospholipase C inhibitor (U73122) significantly reduced release activities in both cell types with a stronger effect in dystrophin-deficient SolC1 (−) myotubes. The same effect was obtained with cyclosporin-A (CsA), known, among other effects, to reduce IP3R-1 expression via calcineurin. Furthermore, cell survival assays demonstrated a protective effect of IP3 pathway blockers in dystrophin-deficient cell lines. These results testify to the involvement of IP3R-1 in enhanced release activities that may participate in the calcium overload observed in dystrophin-deficient myotubes.
Conclusion: Our results suggest that modification of Ca^{2+} release properties, particularly via the IP3 release pathway, are observed in a dystrophin deficient context, suggesting that they could be involved in the Ca^{2+} overload in dystrophic cells leading to cell death. One can think that a partial inhibition of IP3 production could reduce IP3-dependent calcium release and, as a consequence, the calcium-dependent cell death.

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Transglutaminase-dependent rhoa activation and depletion by serotonin in vascular smooth muscle cells
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Introduction: The small G protein RhoA plays a major role in several vascular processes and cardiovascular disorders.
Methods: Here we analyze the mechanisms of RhoA regulation by serotonin (5-HT) in arterial smooth muscle.
Results: 5-HT (0.1–10 muM) induced activation of RhoA followed by RhoA depletion at 24–72 h. Inhibition of 5-HT1 receptors reduced the early phase of RhoA activation but had no effect on 5-HT-induced delayed RhoA activation and depletion, which were suppressed by the 5-HT transporter inhibitor fluoxetin, the transglutaminase inhibitor monodansylcadaverin and in type 2 transglutaminase-deficient smooth muscle cells. Coimmunoprecipitations demonstrated that 5-HT associated with RhoA both *in vitro* and *in vivo*. This association was calcium-dependent and inhibited by fluoxetin and monodansylcadaverin. 5-HT promoted the association of RhoA with the E3 ubiquitin ligase Smurf1, and 5-HT-induced RhoA depletion was inhibited by the proteasome inhibitor MG132, and the RhoA inhibitor Tat-C3. Simvastatin, the Rho kinase inhibitor Y-27632, siRNA-mediated RhoA gene silencing and long-term 5-HT stimulation induced Akt activation. In contrast, inhibition of 5-HT-mediated RhoA degradation by MG132 prevented 5-HT-induced Akt activation. Long-term 5-HT stimulation also led to the inhibition of the RhoA/Rho kinase component of arterial contraction. Our data provide evidence that 5-HT, internalized through the 5-HT transporter is transamidated to RhoA by transglutaminase.
Conclusion: Transamidation of RhoA leads to RhoA activation and enhanced proteasomeal degradation, which in turn is responsible for Akt activation and contraction inhibition. The observation of transamidation of 5-HT to RhoA in pulmonary artery of hypoxic rats suggests that this process could participate in pulmonary artery remodeling and hypertension.

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Myocardial infarction: result of an obstructive athérothrombose
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Introduction: Following an acute coronary occlusion, the first cellular lesions characterize ischaemic necroses of a myocardic zone whose perfusion is suddenly stopped. These lesions extend gradually towards the periphery to lead to a total and final destruction of the ischémic myocardic mass. In 90% of the cases, occlusion responsible for the myocardial infarction is a completely occlusive thrombosis of a principal trunk.
Methods: For our study, we chose a model of experimental diabetes, *Psammomys obesus*. Two batches were exploited: a batch ($n = 12$) subjected to a cocktail of lipids (¼ of egg yolk cuit/j) and a pilot batch ($n = 9$) subjected to the halophilous plants (50 g/j) during 6 months. We analyzed certain plasmatic parameters, evaluated two biochemical markers, carried out a topographic histological study and an analysis of the proliferation of the cardiac myocytes.
Results: Compared to the corresponding witnesses, *Psammomys obesus* showed a remarkable increase in the cholesterolemia, triglyceridemia and glycemia after only 1 month of hyperlipidic diet (1824 ± 663 mg/dL vs. 48 ± 2 mg/dL), (649 ± 423 mg/dL vs. 26 ± 2 mg/dL) and (137 ± 82 mg/dL vs. 43 ± 10 mg/dL). A significant increase in the CPK and troponine I (560 ± 229 U/l vs. 237 ± 101 U/l), (0.09 ± 0.02 µg/l vs. 0.02 ± 0.009) respectively. The morphological examinations of the heart revealed beaches of significant ischaemic necroses and of infarcies zones.
The myocardic cells in culture (early passage), resulting from animals subjected to the hyperlipidic diet show after 72 H of incubation (10^5 cellules/ml), a reduction in half of the rate of their proliferation which reaches 24% vs. 113% their corresponding witnesses.
Conclusion: In conclusion, experimental hyperlipidemy at Psammomys obesus armature of the metabolic deteriorations, marked by: an increase in the cholesterolemia, triglyceridemia and glycemia, a rise in the rate of the troponine I and CPK, a fall of the rate of proliferation of the cardiac myocytes and modifications of the myocardium evoking one ischaemic necrose followed by an infarction localised.

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The influence of hyperhomocysteinemia on blood lipids, lipoproteins profiles and vascular disease in sand rat, *Psammomys obesus*
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Introduction: During the last decades, several clinical and epidemiological studies showed that hyperhomocysteinemia is associated to cardiovascular diseases and is reported to increase the binding of lipoprotein (a). The aim of this study was to investigate whether there is a relationship between homocysteine and Lp (a) levels and vascular disease risk.
Methods: Within the framework of our work, hyperhomocysteinemia is induced in the sands rat *Psammomys obesus* by an intraperitoneal injection of methionine at a rate of 70 mg/Kg of weight corporal/day during 6 months. In parallel, the controls rats receive injections of NaCl with 0.9%.
During the experimentation, we followed the lipids plasmatics parameters evolution (cholesterolemia and triglyceridemia) by colorimetric enzymatic method, homocysteinemia evolution by FPIA method and lipoproteins levels by electrophoresis technique. The histological techniques allowed the histo-morphometric study of the aortic wall.

Results: A moderate hyperhomocysteinemia (20.04 ± 7.77 μM) is observed in the sixth month of treatment against 2.63 ± 2.01 μM at the beginning of the experimentation. Both cholesterolemia and triglyceridemia undergo variations during our experimentation. The analysis of the lipidogrames highlights a significant reduction in the HDL, contrary, VLDL and LDL are increased in the sands Rat subjected to methionine. In addition, we noted with interest the appearance of the lipoprotein (a) after third months of methionine treatment. The vascular wall of *Psammomys obesus* hyperhomocysteinemic is the seat the many focused deteriorations represented by blood aggregations luminales, a hypertrophy of the endothelium and subendothelium, ruptures and unfolding of the internal elastic limiting and of the elastic blades of the media, an accumulation of collagens as well as a change of orientation of the smooth muscular cells media. The rupture of the aortic wall is also observed in the sixth month of treatment.
Conclusion: We conclude that hyperhomocysteinemia altered not only blood cholesterol, lipoprotein profiles but also the structure of the aortic wall.

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CD36 is involved in calcium signalling and phosphorylation of Src kinases induced by linoleic acid in mouse lingual circumvallate papillae
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Introduction: Numerous studies suggest that FAT/CD36 is present in the apical cells of taste buds. Recent evidence has shown that this receptor is implicated in the detection and the perception of fatty acids in the tongue. However, the molecular mechanisms involved in the FAT/CD36 signalling in taste bud cells remain to be explored.
Methods: In this study, we isolated CD36-positive cells from circumvallate papillae of mouse lingual taste buds, by using anti-CD36 antibodies coupled with magnetic beads (Miltenyi kit). The isolated cells were cultured for 24 hours for further experiments. To elucidate the transduction pathways triggered by fatty acids, we employed a long-chain fatty acid, linoleic acid (LA).
Results: We observed that LA induced increases in free intracellular calcium concentrations, $[Ca^{2+}]i$, via the activation of FAT/CD36. Sulfo-N-Succinimidyloleate, an inhibitor of FAT/CD36, abolished LA-induced increases in $[Ca^{2+}]i$. We have also observed that LA induces the phosphorylation on tyrosine residues of Fyn and Yes which belong to the family of Src kinases. Furthermore, the inhibitors of tyrosine kinases (PP2, Genistein and SU 6656) and an inhibitor of SOC (store operated calcium) channels decreased the elevation of $[Ca^{2+}]i$ induced by LA in taste cells.
Conclusion: These results allow us to put forward the hypothesis that LA induces activation of Src kinases and increases in $[Ca^{2+}]i$ in murine CD36-positive cells from circumvallate papillae.

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Role of RYR3 splice variants in calcium signalling in mouse myometrium during pregnancy
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Introduction: Alternative splicing of the RYR3 ryanodine receptor subtype generated a RYR3S short isoform without channel function and a functional RYR3L full length isoform. It has been previously shown that the RYR3S isoform could negatively regulate the native RYR2 subtype in smooth muscle cells as well as the RYR3L isoform when both isoforms were co-expressed in HEK293. Moreover, the RYR3 subtype has been described as a target for cyclic ADP-ribose in several cell types and both expression of ADP-ribosyl cyclase and production of cyclic ADP-ribose have been shown to be increased during pregnancy. Mouse myometrium expresses RYR3, but the role of RYR3 isoforms and their activation by cyclic ADP-ribose during pregnancy have never been investigated.
Methods: The expression of both RYR3 isoforms was specifically inhibited by electroporation of antisense oligonucleotides. The inhibition of RYR3 isoforms was evaluated by RT-PCR, RYR3 immunostaining and westernblot in non-pregnant and pregnant cultured myometrium cells. Calcium signalling activated by caffeine and cyclic ADP-ribose was measured by fluo-4 and laser scanning confocal microscopy.
Results: Here, we show that both isoforms of RYR3 are expressed in non-pregnant and pregnant mouse myometrium. The use of antisense oligonucleotides directed against each isoform indicated that only RYR3L was activated by caffeine and by cyclic ADP-ribose and that RYR3L-mediated Ca^{2+} release was negatively regulated by RYR3S expression in non-pregnant myometrium. At the end of pregnancy, both expression and ability of RYR3L to respond to cyclic ADP-ribose were increased.
Conclusion: Therefore, our results suggest that activation of RYR3L by cyclic ADP-ribose could play an essential physiological role during labour.

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Effects of sub-chronic exposure to 2, 4-Dichloro-phenoxyacetic acid (2,4-D) on parameters of reproductive function in male rats
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The 2,4-dichlorophenoxyacetic acid (2,4-D), a chlorophenoxy herbicide which is widely used for the elimination of weed in agriculture, can get into the environment through effluents and during diverse drainings in relation to its manufacturing and to its transport. In spite of its very recognized toxicity to mammals, its effect on reproductive organs is little estimated. In this work we studied, in adults male rats, the impact of a sub-chronic treatment of 30 days with the 2,4-D on the body growth, the relative weight of testicle, seminal vesicle, prostate and epididymis, parameters of the sperm (number and motility of spermatozoa) as well as on the rat testicular architecture. The serum concentration of gonadotropin (LH and FSH) and steroid (testosterone) was also determinated. Data indicate that the oral administration of the 2,4-D at 100 and 200 mg/kg of body weight (bw) causes respectively a significant decrease of body weight by about 7.6% (226.5 ± 3.9 g vs. 209.2 ± 3.4 g) and 10.5% (226.5 ± 3.9 g vs. 202.6 ± 4.4 g) compared with the control group. Also the 2,4-D induces a significant decrease of relative weight of testicle by about 7.07% (0.591 ± 0.011 g vs. 0.553 ± 0.010 g/100 g) and 7.45% (0.593 ± 0.008 g vs. 0.553 ± 0.010 g/100 g) respectively for 100 and 200 mg/kg bw. The decrease of the relative weight of the prostate is about of 27.82% (0.127 ± 0.006 g vs. 0.176 ± 0.009 g/100 g) and about of 35.35% (0.113 ± 0.0049 vs. 0.176 ± 0.009 g/100 g) respectively with doses 100 and 200 mg/kg of bw. The 2,4-D induces a decrease of the relative weight of seminal vesicle by about 13.80% (0.385 ± 0.015 g/100 g vs. 0.447 ± 0.020 g/100 g) for the dose of 100 mg/kg bw and 26.2% (0.329 ± 0.015 g/100 g vs. 0.447 ± 0.020 g/100 g) for the dose of 200 mg/kg bw. A significant decrease of the relative weight of the epididymis by about 10.31% (0.198 ± 0.004 g vs. 0.022 ± 0.005 g/100 g) for the dose 100 mg/kg bw and 12.18% (0.194 ± 0.003 vs. 0.022 ± 0.005 g/100 g) for the dose of 200 mg/kg bw compared with the control. The histological study of these organs confirms these effects. Light microscopic inspection of testes revealed an active spermatogenesis in the seminiferous tubules of untreated rats showing spermatozoa production. Whereas testes of rats exposed to 2,4-D exhibited a pronounced morphological alteration with loosening of the germinal epithelium and enlarged intercellular spaces resulting from the disappearance of the sertoli cells and interstitial tissue. Spermatozoa are also missing in the lumen of seminiferous tubuli indicating a profound alteration of spermiogenesis processes. Spermatogenesis is also severely affected with a decline of both: – motility by 20.69% (48.5 ± 1.97% vs. 61.2 ± 3.14%) for 100 mg/kg bw and 21.34% (48.11 ± 1.01% vs. 61.2 ± 3.14%) for 200 mg/kg bw and – number by 40.32% ($13.75.10^6$/ml ± 1.4 vs. $23.04.10^6$/ml ± 1.61) and 43.13 % ($13.10.10^6$/ml ± 1.8 vs. $23.04.10^6$/ml ± 1.61) of epididymal spermatozoa respectively for 100 and 200 mg of 2,4-D/kg bw. There is no variation of the level of serum LH and FSH. The concentration of serum testosterone decreased significantly with doses 100 and 200 mg/kg bw.

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How to determine glomerular filtration rate (GFR) before liver transplantation?
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Introduction: Drug-induced chronic renal failure is an emerging problem in liver transplantation all the more so since end-stage liver disease (ESLD) is already associated with renal insufficiency. In this context, the use of creatinine-based estimation of glomerular filtration rate (GFR) has been questioned because ESLD is responsible for a decrease in muscle mass and often associated with jaundice that interfere with the classical Jaffe's method for creatinine dosage. Our aim was to determine (i) if creatinine-based estimation of GFR remains useful in ESLD; and (ii) which methods are the most appropriate to estimate GFR.
Methods: GFR was measured in 35 patients with ESLD using a two-periods renal clearance of inulin after a prolonged distribution time. Results were compared with: (i) measured creatinine clearance based on 3 and 24 h urine collections (CCr 3 h and CCr 24 h respectively); (ii) calculated estimation of creatinine clearance using Cockroft-Gault formula (CG) and of GFR using simplified or complete MDRD formulas (sMDRD and cMDRD respectively). Blood creatinine assays were performed, prior and after plasma ultrafiltration, in the same laboratory by the modified kinetic Jaffe reaction (calibration adjusted to MDRD laboratory). Relationship between GFR and estimates was studied using simple linear regression method. Agreement between methods was determined according to Bland and Altman. Practical impact (classification bias) on NKF (K/DOQI) chronic kidney disease classification was also assessed.
Results: Not surprisingly, the use of CCr 24 h should be avoided because of 24 h urine collection lack of reliability. The strongest correlation with measured GFR is find with CCr 3 h. Preliminary plasma ultrafiltration before creatinine measurement do not provide an obvious benefit on the obtained data relevance. cMDRD is the most precise method and those which exhibits the highest individual predictive value. It is also those which shows the lowest risk of NKF misclassification, included for the lowest GFR values.
Conclusion: Creatinine-based estimation of GFR remains relevant in ESLD population. In these patients, we recommend the use of complete MDRD formula before liver transplantation.

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Hypoglycemic action of two plant extracts in STZ-induced diabetic rats and C57 BLKs db mice
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In Africa, several medicinal plants are routinely used as treatment for diabetes by local doctors. In a trial to rationale this use and with the aim to develop alternative and easily available treatments for diabetes, we investigated the effects of two plant extracts used in traditional medicine on experimental diabetes in rats and mice. Two plant extracts from two different African plants termed A and B respectively are prepared either by organic or aqueous solubilization. Extract A is obtained after solubilization in a mixture CH_2Cl_2/MeOH + 3% DMSO and is administered at the dose of 75 (A75) and 300 mg/kg/day (A300). Extract B is an aqueous solution and is given at the dose of 150 (B150) and 300 mg/kg/day (B300). Both extracts were given by gavage to Wistar male rats made diabetics by a single administration of streptozotocine for 14 days. Non diabetic C57 BLKS db/m were treated both by gavage and by adding the extracts in the drinking water. Administration of both extracts to STZ-diabetic rats resulted in complete correction of hyperglycemia. The values of glycemia (mg/dL) were A_{75} = 108 ± 12; A_{300} = 155 ± 12; B_{150} = 81 ± 4; B_{300} = 120 ± 10 respectively, A_{75}, A_{300}, B_{150} et B_{300} : $P < 0.001$), when compared to untreated diabetic rats (373 ± 7). When given by gavage to non diabetic C57 BLKS db/m, both extracts A75 and B150, decreased glycemia by 52 and 44% respectively. Glycemia was also decreased when extracts were added in the drinking water but a higher dose was required. .Although these strains of mice are not diabetic, their normal glycemia is bordeline (210 ± 9 mg/dL) and finally the treatments normalize glycemia.. Interestingly in STZ rats no body weight lost was observed, moreover the level of triglyceride and cholesterol was significantly decreased. Of interest none of these treatments induced toxic effects. To summarize, both extracts induced reduction of hyperglycemia and partly reduced

lipid abnormality. These results confirm the hypoglycemic properties of these two plants. Additional experiments are in progress to investigate the mechanism of action and the intracellular targets of these extracts and to assess the efficiency of these treatments in type 2 diabetes using BLKS db/db mice.

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Renal localization and expression of tight junction proteins, Claudin-2, Claudin-3, Claudin-5 and Occludin during development in the rat

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Introduction: In several mammalian species, there is a postnatal development of the kidney, both in morphology and function. This is the case in the rat where renal function is not fully established at birth. One of the main tasks for the kidney is the reabsorption of ions and water that occurs through two pathways: a) transcellular route, which depends of several specialized membrane proteins, and b) paracellular route, which depends of the presence of intercellular structures, such as tight junctions and desmosomes. In this work, we investigated different patterns of localization and expression of tight junction proteins (Claudin-2, Claudin-3, Claudin-5 and Occludin) along the nephron and in the renal endothelia during rat development.

Methods: Kidneys from 2, 7, 14, 21 and 60 days old WISTAR rats were perfused and removed to performed frozen tissue sections and immunofluorescence experiments using mono and polyclonal primary antibodies and alexa-488 and 594 secondary antibodies. Proximal and distal localization of proteins was confirmed with aquaporin-1 and desmoplakin-I co-labelling. Fluorescence was examined with a confocal technique.

Results: During kidney maturation, Cl-2 showed a progressive expression and localization in proximal segment of the nephron and the Bowman's capsule with an important cellular reorganization leading to three distinct patterns of localization in the adult rat kidney: (i) in cell border with the classical 'chicken fence' organization; (ii) in brush border membrane probably in S1 segment; (iii) in cytoplasm. By contrast, Claudin-3 showed a definitive pattern of expression and localization (cell border) in distal tubule, renal blood vessels and glomerular capillaries since first postnatal days. Apparently, occludin 'chicken fence' localization along distal tubule and collecting ducts did not change during kidney maturation. In the case of Claudin-5, whereas this protein has been described exclusively in endothelia in the kidney of several species, in rat Claudin-5 was also expressed and located in terminal segment of the nephron (distal tubules and collecting ducts) showing a linear pattern at the border of renal cells since first postnatal days.

Conclusion: This work demonstrated that a specific reorganization of renal distribution of tight junctions proteins occurs during rat development. Moreover, for the first time, the expression of Claudin-5 in the terminal segments of the nephron was evidenced in rat. Supplemented by funcional studies, these data will allow a better understanding of the relationship between the processes of maturation of kidney tissue and the paracellular transport along the nephron.

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Barin growth in autism: first study of evidence of prenatal overgrowth

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Introduction: Autism is a complex neurodevelopmental disorder of whose etiology remains unknown. Evidence from family studies and genetic linkage studies strongly suggests a genetic component to the disorder. However, evidence also points towards environmental factors. A great deal of research and discussion has focused on whether brain and head size are abnormal in autism. Recent meta-analysis of studies of autism head circumference (HC), magnetic resonance imaging (MRI), and post-mortem brain weight (BW) reveal a period of pathological brain overgrowth in the first years of life and then abrupt cessation. It is hypothesized that the rapid growth will differentially affect large, integrative neurons that normally require protracted maturation, for example neurons within the later maturing frontal cortex. While it is unclear what triggers this postnatal brain overgrowth, it is clear that knowing the underlying mechanism and timing (pre-, peri-, or post-natal) of this trigger is crucial. Here we report the first study of prenatal measurement of brain growth in autism.

Methods: Fetal echographic biometric parameters were analysed in a retrospective study. Parameters were available for 46 fetus later diagnosed with autism. Biparietal diameters of the first, second and third trimester of gestation were compared.

Results: Biparietal diameter Z scores relative to norms did not differ between first and second trimester of gestation but significantly increased from second to third trimester.

Conclusion: These results suggest that the period of exceptionally rapid head growth begins not in the first years of life but before birth. Dysfunction of myelinization and synaptogenesis occuring at this period might be a part of neurobiological processes underlying autism. Furthermore between all genes involved in brain development, those with late expression during gestation should be better candidate genes.

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Time course of the M response and H reflex on rat soleus muscle after lidocaine tibial nerve's block

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Introduction: Lidocaine block is often used to evaluate patient with spastic equinus foot, as a test of the clinical impacts of a selective neurotomy. The application of lidocaine at the human soleus nerve generates a strong decrease in the M wave maximal peak to peak amplitude. Furthermore this so-called 'motor block' also decreases the H reflex amplitude and seems even more efficient on sensory afferences than on motor collaterals. This effect of lidocaine on the reflex pathway has never been explained in the literature. To better understand it, we propose an animal study of the time course of H reflex and M wave after lidocaine block.

Methods: The seven adult wistar rats included in this preliminary study were tested bilaterally. The procedure was done under general anesthesia after intra peritoneal injection of ketamine witch not modify the reflex pathway. Rats were in lying position on the experimental table with the studied hind limb maintained in standardized position (knee and ankle flexed at 90°). The lidocaine (0.5 mL) was injected percutaneously after anatomic landmarks were done to insert the needle in contact of the tibial nerve. Before lidocaine injection, the stimulus intensity and electrodes location were chosen to obtain both M and H responses as previously reported in the rat. Ten M and H responses were recorded before lidocaine injection. After the injection, the stimulus was applied every minute and until a complete recovery of control response.

Results: The mean latencies were 1.76 ms (+/-0.5 ms) for M response, and 7.74 ms (+/-1.1 ms) for H reflex. The mean durations were 5.51 ms (+/-0.6 ms) for M response, and 3.85 ms (+/-0.3 ms) for H reflex. The mean maximal peak to peak amplitudes were 36.4 mV (+/-10 mV) for M response, and 13.7 mV (+/-7 mV) for H reflex. There were no modifications on latencies and on durations of H and M responses after lidocaine injection. A stimulus was applied immediately after the injection and, at that time, M wave and H reflex were not modified. At the following stimulus, one minute later, the M wave was around 53% (+/-25%) of its control value, and the H reflex was abolished. The time course of the M wave and H reflex recovery was identical and the mean delay for total recovery was 117 minutes (+/-20 min).

Conclusion: This animal model of lidocaine effect on the direct motor response (M wave), and on the reflex amplitude (H reflex) confirms that lidocaine seems to be more efficient on the sensory spindle afferents than on the motoneurons. The lidocaine blockage therefore appears very interesting in spasticity assessment. Complementally experiences are in progress to better understand the mechanisms of lidocaine action.

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An event-related potential study of cognitive maturation

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Introduction: Current study was aimed at investigating the usefulness of event-related potentials in evaluating cognitive maturation.

Methods: The P300 and N400 components were recorded from 28 electrodes in 80 children divided into four age-matched groups: 20 gifted children, 20 children with attention-deficit/hyperactivity disorder, 20 children with Down syndrome, and 20 controls. All the participants were right-handed ranged from 8 to 12 years. The P300 was elicited using the classical oddball paradigm in both visual and auditory modalities, and the N400 during a semantic relation judgment task in the visual modality.

Results: Gifted children showed higher visual P300 and N400 amplitudes than other groups, without significant differences from controls in latencies. No differences were observed in the auditory modality between gifted children and other groups. The P300 latency was longer in children with attention-deficit/hyperactivity disorder than in controls for the visual modality, especially in the frontal region, with smaller amplitude in the auditory modality, without N400 differences. For all the event-related potentials, children with Down syndrome presented longer latencies and smaller amplitudes than other groups. The P300 latency decreases as a function of age in both modalities without significant amplitude modification in three groups. Only children with attention-deficit/hyperactivity disorder demonstrated a positive correlation of the auditory P300 amplitude to age. P300 parameters were not correlated to the intellectual quotient, except in the attention-deficit/hyperactivity group with a negative correlation to the P300 latency in both modalities. The N400 mean amplitude decreases as a function of age in all the groups with a positive correlation to the intellectual quotient in three groups. No significant correlation was found in the Down syndrome group, probably because of a limited validity of the Wechsler scale in this population.

Conclusion: Present findings argue for particularities in visual information processing in giftedness and favour the hypothesis of a frontal dysmaturation in attention-deficit/hyperactivity disorder. P300 and N400 parameters reflect attention and memory disabilities in Down syndrome. This study underlines the usefulness of both P300 and N400 components in investigating cognitive maturation.

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Assessment of energy demand in the new Olympic windsurf board: Neilpryde RS: X®

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Introduction: The aim of this study was to evaluate the energy demands of sailing the new Neilpryde RS: X® Olympic windsurf board.

Methods: Ten skilled male subjects performed an exhausted incremental treadmill test to determine their maximal physiological parameters. Thereafter, four tests in a randomed order were performed using two wind conditions, light [2–4 ms^{-1} (4–8 knots)] and strong: [9–11 ms^{-1} (16–22 knots)]. Oxygen consumption (VO_2, $ml.min^{-1}.kg^{-1}$), blood lactate concentration ($[la]_b$, $mmol.l^{-1}$), and time spent for pumping (% total time) were recorded during 10 minutes of up-wind leg and during 6 minutes of down-wind leg.

Results: Results indicate that sailing on RS: X is associated with a high level of energy demand using both aerobic and anaerobic pathways whatever the wind conditions. During down-wind leg, VO_2 ($ml.min^{-1}.kg^{-1}$), $[la]_b$ ($mmol.l^{-1}$) and time spent for pumping (% total time) values were for the light and strong wind conditions, 56.5 ± 5.9 vs. 55.5 ± 3.6; 10.2 ± 1.5 vs. 9.6 ± 2.3 and 69 ± 5% vs. 64 ± 2%, respectively. In contrast, during up-wind leg the same parameters for light and strong wind were 53.9 ± 4.5 vs. 40.4 ± 7.2; 9.7 ± 2.8 vs. 5.0 ± 2.7 and 66 ± 3% vs. 37 ± 8%, respectively.

During up-wind leg with strong wind conditions less time was spent in pumping ($P < 0.05$), mean oxygen consumption values were close to 60% VO_{2max}, and post exercise blood lactate was lower than 50% maximal lactate concentration. These results could be related to the time spent in pumping action, involving the whole body activity.

Conclusion: When sailing with the RS: X board, physiological demand seems to be higher than with the previous official Olympic windsurf board [mistral one design® (MOD)]. This difference could be mainly attributed to the specific biomechanical constraints induced by each board characteristics.

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Physical training and evoked potentials: neurophysiological particularities in sports involving vision

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Introduction: Present study was aimed at further tracking neurophysiological particularities in sportsmen using evoked potentials, and more specifically at assessing how sports involving vision influence evoked potential patterns.

Methods: Twenty sportsmen and ten sedentary subjects participated to this study. Sportsmen were divided into two groups: ten tennismen and ten shooters. All the subjects were healthy right-handed men aged from 18 to 35 years. The electrophysiological investigation included: visual evoked potentials, brainstem auditory evoked potentials, and P300 recordings. Sensory evoked potentials were elicited using standard protocols, by recording responses to a checkboard reversal pattern and to clicks. The P300 component was elicited using the classical oddball paradigm in both visual and auditory modalities.

Results: In the visual modality, sportsmen differ from sedentary subjects by shorter latencies at both sensory and cognitive levels (P100 and P300 components, respectively). In the oddball paradigm, shorter latencies were also observed in sportsmen for earlier components: N1, P2 and N2. Tennismen demonstrated shorter visual P300 latencies than shooters, without latency differences in the P100 or perceptual components (N1 and P2). No significant differences were found in amplitudes between sportsmen and sedentary subjects neither for visual evoked potentials nor for the visual P300 component. In the auditory modality, only tennismen differ from sedentary subjects with shorter latencies in brainstem auditory evoked potentials (for the I-V interwave interval, and more specifically the III-V interwaveinterval) and also shorter P300 latencies. No significant differences were showed between sportsmen and sedentary subjects in amplitudes neither for brainstem auditory evoked potentials nor for the auditory P300 component.

Conclusion: Current study suggests that sports involving static and dynamic vision might influence electrophysiological data in the visual modality in different ways at cognitive level, without differences at sensory and perceptual levels. Moreover, tennis is also associated to electrophysiological particularities in the auditory modality since the latter is critical in this sport. Present findings argue for neurophysiological particularities in sportsmen related to sensorial skills and cognitive abilities needed by their physical training.

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Skeletal muscle mass recovery after notexin injury: positive effects of running exercise

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Introduction: Skeletal muscle is able to regenerate after extensive injury (notexin injection) probably through fast activation and proliferation of satellite cells. Then new myocytes differenciate into myotubes and express myogenin and immature MHC isoforms. During maturation, myotubes fuse to form mature myocytes with peripherical nucleus and expression of mature MHC isoforms. The recovery of muscle mass takes more than 40 days in sedentary animals. When physical activity is decreased (28 days suspension during recovery), the regenerated muscle mass is also decreased. In the present study, we tested the hypothesize that increased muscle activity could lead to a faster recovery of skeletal muscle mass after extended muscle injury induced by myotoxin injection.

Methods: Left soleus muscles of 96 Wistar rats were injected with Notexin and right ones were kept intact, as control (D0). Three days after injury, half of the animals began running exercise: calibrated exercise on treadmill associated with voluntary exercise in special cages (exercised animals, E). The other half was kept in standard cages (sedentary animals, S). They were sacrificed at days D5, D7, D14, D21, D28 and D42 post injury. Both soleus muscles were removed (intact and injured), weighed and immediately frozen in liquid nitrogen. HES coloration were performed to evaluate muscle recovery and quantify fiber cross sectional area (FCSA). An indicator of satellite cells activation (PCNA), expression of myogenic regulatory factors (MRF, MyoD and Myogenin), markers of the MAPK pathway (p38, ERK-1/2 and JNK phosphorylated and total forms) and markers of the main signalling pathways involved in muscle mass control (Akt, mTOR, 4E-BP1 and p70 phosphorylated and total forms) were studied by Western Blot.

Results: In sedentary animals, soleus weight is recovered at D42 after injury in S rats, whereas it is recovered at D21 in E animals. At D21, FCSA values of injured soleus myofibers were significantly higher in E than in S muscles (+58%, $P < 0.05$). PCNA and MyoD expression increased in regenerating muscles, but whereas the values are the same as in non-injured soleus at D14 in S muscle, they recovered their control levels at D21 in E muscles. Myogenin expression is increased with injury in E and S groups, without any effect of exercise. MAPK phosphorylation is also increased with injury. Whereas p38 phosphorylation is recovered more quickly in E muscles (till D14 instead of D21), ERK-1/2 phosphorylation in E muscles is longer (till D21 instead of D14) and JNK phosphorylation seemed to be less important at D7 in E muscles. Akt phosphorylation is increased in a same extent in both regenerating muscles. Compared with S levels, mTOR and 4E-BP1 phosphorylation is more important at D14 in E soleus, and returned to control values more quickly (D21). p70 phosphorylation is increased after injury, without any effect of exercise.

Conclusion: Running exercise after a notexin injury leads to a rapid recovery of muscle mass. It likely allows a more important muscle satellite cell proliferation. Furthermore, the increased phosphorylation of mTOR may induce a greater phosphorylation of 4E-BP1, leading to a greater eIF-4G complex formation and thus to a more efficient ARNm translation, resulting in the increased FCSA in exercising injured muscle.

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Impact of acute exercise on QT dispersion

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Introduction: Exercise tests are usually used for diagnosis of cardiac abnormalities. ST segment depression reflects myocardial ischemia and ventricular repolarization dispersion is used as a risk factor. The aim of this study was to evaluate the kinetics of QT dispersion (QTd) during exercise and recovery from 12-lead electrocardiogram depending on ST depressions (STd).

Methods: Thirty six patients performed a treadmill incremental test followed by 6-min of recovery with 12-leads ECG recording each minute. Three groups of patients were defined according to STd criteria: Acute (STd ≤ -4 mm.), Positive (STd ≤ -1 mm.), Control (high risk factor patients without STd). Ventricular repolarization modifications were assessed by QTd, standard deviation of the 12-leads and QTd normalized by QT mean.

Results: All parameters of ventricular repolarization increased significantly according to exercise level ($P \leq 0.0001$) without significant differences between groups. Moreover, all ventricular repolarization parameters decreased deeply during recovery ($P \leq 0.001$). Finally, at the end of recovery the values of ventricular repolarization were shorter than during the resting period ($P \leq 0.001$).

Conclusion: Acute exercise induced a sharp decrease of QTd during the recovery whatever the ST segment depression. These results may explain the positive impact of exercise training on QTd.

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Does the VO_{2max} value predict the formation of intravascular circulating bubbles during decompression of healthy divers?

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Introduction: Objective: To study a possible correlation between the individual VO_{2max} value and the quantity of intravascular bubbles formed at the end of a saturation dive.

Methods: Study protocol: Forty-two males took part in this study. At least one week prior to the experimental dive, each subject underwent an incremental maximum test to determine maximal oxygen uptake (VO_{2max}) on a cycloergometer. The divers had been told to avoid any physical exercise during the 48 hours prior to the dive. The subjects were divided into two sub-groups. Sixteen of them completed a dive in a tank and 26 in the sea. The two dives had the same profile: 30 minutes at 30 metres with identical ascent protocols. The age, Body mass index and the VO_{2max} values of the two sub-groups were similar (respectively 33.3 ± 3.7 years; 24.1 ± 1.5and 51.7 ± 8.1 ml $kg^{-1}.min^{-1}$in the case of the divers in the tank V 37.8 ± 7.5 years; 24.5 ± 2.1 and 48.9 ± 4.5 ml $kg^{-1}.min^{-1}$ for those diving in the sea). Circulating venous bubbles were detected using a pulsed Doppler together with a 2 MHz probe. The data was recorded 30, 60 and 90 min. After emersion.

Results: Bubble formation in both types of dive was significantly correlated to the age and Body mass index of the divers. On the other hand, there was no significant relationship between the VO_{2max} values and bubble formation for the two sub-groups.

Conclusion: As age simultaneously influences the VO_{2max} value and bubble formation, the choice of a homogenous population in terms of age, unlike previous studies, allowed us to observe that bubble formation is not linked to the VO_{2max} value. Nevertheless, it is still true that VO_{2max} reflects a subject's level of physical activity, which is known to influence bubble formation.

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Effects of acute exercise and exercise training on cognitive functions among patients with cardiac diseases

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Introduction: A lot of studies investigated the effect of physical activity on cognitive performance in healthy subjects, but no consensus was established. In cardiac diseases, which have been associated to a cognitive impairment, research about cognition improvement assumes a clinical interest.

We assessed the effect of both acute exercise and aerobic training on cognitive functions among patients with CAD and HF participating to a cardiovascular rehabilitation program.

Methods: Twenty-four men (mean age = 51.6 ± 6.5 years) participated to the study. They completed two experimental sessions, before and after exercise training. During each session, they underwent cognitive evaluations at rest, and during exercise on cycloergometer (30% of maximal power). Two kinds of cognitive evaluations were used: an exclusively cognitive test (arithmetic test, memory test …) and a tracking task, including motor precision.

Results: Acute exercise improved tracking performance in the first experimental session (before exercise training). After exercise training, acute exercise also improved cognitive performances. Exercise training improved both performances at rest, but only cognitive performances during exercise and finally optimised the positive effect of acute exercise on cognitive performances.

Conclusion: In a population with cardiac diseases, limits exist concerning the positive effect of acute exercise on cognitive functions. However, this study provides evidence for improvement of cognitive functions with cardiac rehabilitation training program.

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Bone tissue in young boy soccer players: relationship with hormonal parameters and biochemical markers at different pubertal stages

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Introduction: The aim of this study is to determine soccer practice effect on bone tissue and hormonal parameters at different pubertal Tanner stages.

Methods: A total of 134 boys are recruited from different schools and Tunisian soccer clubs. The subjects were divided into two groups. Soccer players group was constituted of 93 subjects that practice soccer 11 months in a year, for at least 3 yr (3.9 ± 0.8) at the rate of 4 times a week. The control group was formed by 43

sedentary subjects. The Tanner stage was determined and recorded by an endocrinologist according to the method of Tanner. Total body and regional measurements of bone mineral density and bone mineral content, fat and lean mass were made using dual-energy x-ray absorptiometry. Blood samples were obtained from the participants between 0800 and 0930 am following an overnight fast. The blood samples were used to measure concentration of bone markers and growth hormones.
Results: Bone mineral density and content for the whole body, lower limbs, pelvis, and femoral neck were higher in all boy soccer players than in control subjects ($P < 0.001$). The bone mineral density and the bone mineral content differences are significant for the early and late puberty groups ($P < 0.001$) for all body parts except for lumbar spine BMC in early puberty. Nevertheless, in the prepuberty stage, there was only a significant difference between soccer players and controls in spine BMC ($P < 0.05$). The differences of serum IGF-1, IGFBP-3, GH and total Testosterone between pubescent soccer players in early and late puberty are significant when compared with controls ($P < 0.05$). However no significant difference was observed in the prepuberty stage. A significant correlation was found between BMD and serum IGF-1, IGFBP-3, and GH for soccer players but none for the control group.
Conclusion: This study provides that there is a relationship between the development in bone mass, hormonal concentrations and physical activity at early and late puberty in Tunisian pubescent soccer players. The correlation reported between hormonal concentrations and BMD, implies that this development of the BMD may be linked to an improvement of the IGF-1, IGFBP-3, and GH throughout puberty in pubescent soccer players. Furthermore, the enhancement of BMD and hormonal concentrations, more markedly at late puberty, is due to the effects of soccer participation.

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Exercise calorimetry with 6 min steps closely predicts the lipid oxidation flow rate of a 45 min steady state targeted training session at the level of maximal lipid oxidation (LIPOXmax)
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Introduction: Exercise calorimetry allows the assessment of the balance of substrates at various levels of exercise and thus the targetting of training on metabolic bases. We aimed at investigating whether the maximal flow rate of lipid oxidation predicted with this test fits with that which can be measured during a 45 min steady state work load conducted at the corresponding power intensity as done during targeted training protocols.
Methods: We determined the point of maximal oxidation of lipids (LIPOXmax) by calorimetry with a protocol using 6 minutes steady state workloads. The flow rate of lipid oxidation determined at the level of this LIPOXmax was compared with the flow rate of lipid oxidation over 45 minutes of steady state exercise (cycling) at the power corresponding to this LIPOXmax. 11 sedentary subjects (age 49.2 ± 4.9 years; BMI: 29.4 ± 1.74 kg/m^2) performed these two measurements.
Results: The LIPOXmax occurs between 11 and 71% of the theoretical Pmax (average 29.58% ± 5.42%) and calorimetry predicts at this level a flow rate of lipid oxidation averaging 147.2 ± 16.1 mg/min. The flow rate actually measured during the 45 min workload averages 151.7 ± 11.6 mg/min, *i.e.*, a total of 6.8 ± 0.5 g over the 45 minutes. This flow rate is predicted by the calorimetry with an average deviation of 4.51 ± 8.7 mg/min on the Bland-Altman plot and a satisfactory correlation ($r = 0.855$ $P < 0.001$). Furthermore the graded calorimetry test predicts the heart rate over the 45 minutes with a mean difference of 7.5 ± 4.06 beats/min.
Conclusion: Thus, the 6-min steps procedure closely predicts the actual rate of lipid oxidation that would be measured over a 45 min training session and is an accurate procedure for targeting training on a metabolic basis.

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VO_2 kinetics and bronchial hyper-responsiveness in professional cyclists
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Introduction: The relation between oxygen consumption (VO_2) and work rate (W) is classically considered to be linear (Astrand). Recently, the comparison of the VO_2 measured during an incremental test with the extrapolated values from the VO_2/W relation below the lactate threshold, can show an excessive VO_2 in healthy subjects (Zoladz) or a reduced VO_2 in professional cyclists (Lucia). A high prevalence (50%) of bronchial hyper-responsiveness has been observed in professional cyclists (Medelli). Clinical investigation, lung function testing at rest or during exercise and pharmaceutical tests are not always discriminatory enough for a diagnosis. Could the analysis of VO_2 kinetics below and above VT provide additional information?
Methods: A total of 37 professional cyclists (27 ± 4.1 years, 179.7 ± 6.8 cm, 71.2 ± 6.8 kg, VO_{2max}: 67.55 ± 5.9 mL.kg^{-1}.min^{-1}, Wmax: 415 ± 35 Watts) were separated in two groups characterized by positive (BHR+, $n = 18$) or negative (BHR−, $n = 19$) bronchial hyper-responsiveness according to the following parameters: clinical investigation, lung function testing at rest and after exercise, and methacholine challenge. Subjects performed an incremental test (50 W/3 min) until exhaustion on a bike ergometer (Lode Excalibur). Physiological parameters were measured continuously (Jaeger Oxycon V) and recorded every 30 s until test termination. The values of the last minute of each completed step were averaged and used for regression analysis below and above the ventilatory threshold with calculation of slopes and intercepts.
Results: Except age (29.2 ± 3.4 vs. 25.3 ± 3.9 for BHR− and BHR+; $P < 0.05$), there was no significant difference between groups regarding anthropometrics, physiological and lung function testing data. However, during the incremental test the VO_2 relation of the BHR+ group exhibited a breakpoint with a steeper VO_2/W slope above compared to below VT (0.497 ± 0.031 vs. 0.561 ± 0.051 respectively, $P < 0.0001$) while the VO2/W relation was linear in the BHR− group. The VO_2/W slope above VT was significantly higher in BHR+ compared to BHR− ($P < 0.05$). These results were unchanged when taking into account age effect.
Conclusion: In this group of professional cyclists, lung function testing cannot always differentiate between subjects according to their BHR status. However, it appears that the VO_2 kinetics during an incremental exercise test can be a discriminatory parameter contributing to the diagnosis of BHR.

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Metabolic and hormonal responses to starvation-induced hyperactivity in rats: clues on anorexia athletica
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Introduction: In order to maintain low body weight, many athletes use pathogenic methods, putting them at greater risks of sustaining eating disorders (Eds) than the general population. Even though numerous causal factors have been suggested, no consensus has yet been reached on the physiological origins of this syndrome. An animal model is available to investigate physiological responses to AA: Food restricted rats which are given free access to a running wheel paradoxically tend to increase running activity while reducing food intake. Low T3 syndrome, hypercorticosteronemia and hypoleptinemia seem implicated in this paradox, however, the data are under debate. Aim of this study was to analyze metabolic and hormonal responses to exercise, food restriction, and starvation-induced hyperactivity. Corticosterone and hormones regulating appetite and food intake could be simultaneously involved in hyperactivity and decreased food intake.
Methods: A total of 56.6 weeks old wistar rats were divided into four groups: WAL (Wheel, Ad Libitum), WR (Wheel, Restricted), SAL (Sedentary, Ad Lib), SR (Sedentary, Restricted). Restriction consisted in reducing food access to 75 minutes per day. During this time frame, animals had no access to the wheel. After 7 days of food restriction, rats were sacrificed by anaesthetic (pentobarbital) and aortic puncture. Last day fasting plasma glucose, urea, fatty acid, insulin, leptin, ghrelin, and corticosterone were measured.
Results: Paradoxically, despite a marked increase in running activity, we did not see any reduction in total food intake in our starvation-induced hyperactivity rat model. SR weight decreased of 23.5% whereas WR decreased by 27.5%. Wheel running increased by more than 600% in WR compare to WAL. Glucose, insulin and IGF-I concentrations decreased by 15%, 109% and 233%, respectively ($P < 0.001$) in WR compare to SAL. Leptin was greatly decreased, and fell to undetectable levels, whereas ghrelin increased by 394% ($P < 0.001$). Corticosterone levels were no different between the groups. Adrenal weight was lower in restricted rats showing a decrease in HPA axis activation. In WR, urea was increased by 56% ($P < 0.001$).
Conclusion: Our results are in contradiction with previous studies on older rats. The absence of prefeeding corticosterone peak in WR was likely to prevent further protein utilization. Indeed, in WR urea increase reflecting greater protein utilization in restricted rats.

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Muscle metabolism in patients with exercise intolerance
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Introduction: Many patients complain of muscle pains, fatigue and exercise intolerance. The investigation of such diseases underlies the need of an integrated physiological approach and is quite complex.
Methods: In this study, 136 patients complaining of such symptoms performed a maximal exercise test, followed by a muscle biopsy for oxygraphic and histologic analyses. Vastus lateralis muscle biopsies were taken by the percutaneous Bergström technique after local anesthesia (Xylocaine). *Mitochondrial respiration* was studied *in situ*, using a Clark electrode (Strathkelvin Instruments, Glasgow, UK). Measurements were carried out at 30°C with continuous stirring in 3 ml of the oxygraph solution with different respiratory substrates (glutamate, pyruvate, assessment of the different complexes of the respiratory chain using activators and inhibitors). Evaluation of muscle biopsies by histochemical staining used standardized methods (NADH-TR, SDH, COX).
Results: The main results of the study were as follows. The ADP-stimulated maximal respiration under glutamate and under pyruvate feeding were significantly correlated to maximal oxygen uptake during exercise test ($r = 0.26$, $P = 0.004$, $r = 0.20$, $P = 0.037$, respectively). The comparison of the two approaches on muscle biopsies, *i.e.* the functional one by polarography, and histo-enzymological one, showed a global degree of concordance of 76.9%, which is very satisfactory. The analysis of discordant cases was of special interest, and showed that the two approaches are complementary in a view of an integrated investigation.
Conclusion: In summary, exercise intolerance is a complex pathophysiological field, which needs an overview of the major biochemical pathways of energetic metabolism in human skeletal muscle.

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Physical capacity of subjects with sickle cell trait during long duration muscular exercise in hypohydration
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Introduction: The physical and physiological behavior of the subjects with sickle cell trait (HbAS) is somewhat sporting ambiguity during exercise requiring in intense and long duration efforts whereas this genetic abnormal character, is generally regarded as being a benign disorder. Sickle cell is a recessive hereditary disease which can induce systemic vaso-occlusive phenomena which do not save the kidney. The aim of this study was to analyze the cardiovascular and thermoregulatory parameters during exercise with hypohydration in tropical climate of normal subjects (HbAA) and subjects with HbAS.
Methods: Twelve experimental subjects HbAS and l2 control subjects HbAA were selected on the basis of the test of Emmel and electrophoresis of haemoglobin. In HbAS subjects, the rate of haemoglobins S was on average 40.1% ± 3.36, that of haemoglobin A_1 of 57.4% ± 4.05, and of haemoglobin A_2 of 2.7% ± 1.02. The age of these subjects was on average of 25 years ± 2,13, their weight of

67,125 kg ± 6,68 on average and their size of 177 cm ± 5.90. The age of subjects with HbAA was average of 25 ans ± 2.02, their weight of 67.21 kg ± 4.48 on average and their average size was of 177 ± 4.95.
These subjects carried out a test of effort on ergometric bicycle of intensity corresponding to 85% of the theoretical maximum heart rate (220-age ± 10) during 1heure and in situation of hypohydration. The ambient temperature was 26°C with a percentage of humidity of the air between 65 and 68%. The urinary concentration of ions, sodium (Na^+) and potassium (K^+) was measured before and after exercise.
Results: Haematological analysis has shown any difference between the two groups. The average values of the heart rate and the blood pressure, measured, at rest, during the exercise, after three minutes and five minutes of recovery, are comparable between the two groups of subjects. The thermoregulatory data present a significant increase in the rectal temperature ($P < 0.005$) during exercise in subjects HbAS. The urinary ions had not presented any significant difference in the Na+/K+. One subject HbAS had a Na+/K+ superior at 1 and presents probably an organic renal insufficiency.
Conclusion: This study shows that during a constant muscular exercise of one hour in situation of hypohydration and at 26°C of ambient temperature, the subjects with sickle cell trait present an aptitude for the physical exercise and thermoregulation comparable to normal subjects, at rest and after three and five minutes of recovery.

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Determination of caloric contribution and study of deviation of the food behavior in 300 teenagers high school students

R Aouadi[a] [a]*Tunis - Tunisie*

Introduction: The incidence of obesity has trebled in the last 20 years and in Tunisia 9–36% of women are now obese (Body mass index > 30). It is thought that obesity is a direct result of changes in our environment including the advances in technology through motorised transport, automation, home screen entertainment and easier access to calorie rich foods at a lower cost. Evidence is mounting that a reduction in levels of physical activity is a major factor in this trend. Physical activity has been shown to aid recovery from heart disease. The benefits of exercise on heart health can be felt at even moderate levels of activity with the greatest benefits being seen when sedentary individuals become moderately active. The purpose of this study was to investigate the influence of physical activity on body weight, maximal oxygen uptake (VO_2 max) heart rate in sedentary volunteers women aged from 20 to 35 years.
Methods: Thirty-nine sedentary volunteers subjects (women), were distributed in two groups. The subjects of the first group (G1, $n = 23$) were aged from 20 to 26 years and weighed 62.41 ± 10.76 kg; those of the second group (G2, $n = 16$) were aged from 27 to 35 years and weighed 67.21 ± 9.61 kg. All subjects had to train during 8 weeks (3 days per week and for 1 h per day) in three differents deprived sport halls, with a physical aerobic training program. At the beginning (t_0) and at the end (t_8) of the experimental protocol, the subjects of groups G1 and G2 were submitted to several tests allowing to follow the evolution of some physiological and anthropometric parameters (body weight, heart rate, arterial pressure, VO_2 max and to evaluate the physical aptitude (step-test, test of Crampton).
Results: The results showed that aerobic physical activity for 8 weeks reduce the body weight only in group G2 wihout significant modification in their VO_2 max. However, our findings demonstrated that the subjects of group G1 performed significantly their VO_2 max but wihout significant modification in their body weight (the group G1 was with low initial levels of body weight). In parallel the condition physique were enhanced between the pre-test and the post-test in the two groups G1 and G2. In addition, we found that a progress has been noted in the resistance to the fatigue in groups G1 and G2 but without significant modification in their heart rate or arterial pressure.
Conclusion: In conclusion, the results showed that the aerobic physical activity during 8 weeks in sedentary women increase VO_2 max, enhanced the physical condition, reduced slightly the bodily weight but without modifying heart rate or arterial pressure.

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The relevance of the classification of groups of teenage sprinters

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Introduction: The process of maturation implies cumulative changes to the level of the capacities motor, cognitive and physical of the person. Among these physical capacities, the exploding strength and the speed of displacement of the body represent two determining factors of the performance in sprinters. During the period of adolescence, the athletes undergo the biologic maturation process. However, according to previous works, the evolution of this process varies from a person to another. Biologic maturation through the pubertal stages on the exploding strength and the speed of displacement at teenage sprinters. On the other hand, we wanted to demonstrate the relevance of the classification of the groups of athletes according to the pubertal stages in relation to the classification of the groups (minimal cadet) that takes as a basis on the chronological age.
Methods: The total sample of our study is composed of 43 runners of speed (aged from14 to 17 years) of which 22 athletes belonging to the minimal category (aged from 14 to 15 years) and 21 athletes belonging to the category cadet (aged from 16 to 17 years). These athletes have been distributed according to their biologic ages through the pubertal stages to Tanner. Our sample has been submitted to two physical tests: a test of vertical jump and a test of speed on a distance of 30 m.
Results: Our data showed that the 22 minimal athletes, aged from14 to 15 years, were distributed according to the stages to Tanner as five athletes in stade2, eight athletes in stade3 and nine athletes in stade4. With regard to, the 21 athletes cadets, aged from 16 to 17 years, they were distributed as three athletes in stade3 and 18 athletes in stade4. The evolution, according to the biologic age, of the qualities of the exploding strength and the speed of displacement showed that was no significant difference between two successive pubertal stages. Indeed, we haven't noted a significant difference between the stades2 and 3 nor between the stade3 and 4. However, we noted a significant difference ($P < 0.05$) between the stades2 and 4.
Conclusion: In conclusion, our results showed that on the one hand, the biologic maturation has a significant effect on the evolution of exploding force and on the speed between the stade2 and the stade4. On the other hand, the classification of the groups of athletes in the period of adolescence is more applicable according to the pubertal stages rather than by the chronological age. Our recommendations to the trainers, in the classification of the groups athletes, consist to avoiding to be content with the federal classification (minim, cadet) and to take in consideration the biologic age.

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Physical activity effects on body weight, VO2 Max and heart rate

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Introduction: The incidence of obesity has trebled in the last 20 years and in Tunisia 9–36% of women are now obese (Body mass index > 30). It is thought that obesity is a direct result of changes in our environment including the advances in technology through motorised transport, automation, home screen entertainment and easier access to calorie rich foods at a lower cost. Evidence is mounting that a reduction in levels of physical activity is a major factor in this trend. Physical activity has been shown to aid recovery from heart disease. The benefits of exercise on heart health can be felt at even moderate levels of activity with the greatest benefits being seen when sedentary individuals become moderately active. The purpose of this study was to investigate the influence of physical activity on body weight, maximal oxygen uptake (VO_2 max) heart rate in sedentary volunteers women aged from 20 to 35 years.
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Conclusion: In conclusion, the results showed that the aerobic physical activity during 8 weeks in sedentary women increase VO_2 max, enhanced the physical condition, reduced slightly the bodily weight but without modifying heart rate or arterial pressure.

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Lipid mobilization: a limiting factor of lipid oxidation in trained men

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Introduction: The rate of fat oxidation (FOx) in human skeletal muscle may depend on the ability of muscle to uptake and oxidize fatty acids, as well as on plasma fatty acid availability. It has been shown that lipid oxidation was increased the day after following a single exercise bout. The aim of this study was to investigate the relationship between increased FOx and lipid mobilization during exercise and to determine how sucrose ingestion might affect these parameters.
Methods: Eight endurance trained men (age: 25.4 ± 5.8 y; fat mass: 10.4 ± 2.5%; VO_2max: 74.6 ± 4 $ml.min^{-1}.kg^{-1}$ lean body mass) exercised for 90 min at 70% VO_2max after an overnight fast. They exercised 45 min in fasted condition (CON, control), then ingested a bolus of sucrose (0.75 $g.kg^{-1}$) and carried on exercising for 45 min more (CHO, carbohydrate). The experiment was performed either after 36 hours (No Exercise: *NEx*) or 18 hours (Exercise: *Ex*) without prior exercise. Lipid mobilization was studied by microdialysis in abdominal subcutaneous adipose tissue (dialysate glycerol concentrations were taken as index of lipolysis). FOx ($g.min^{-1}$) was measured by indirect calorimetry.
Results: At rest, lipid mobilization and FOx were significantly higher in *Ex* compared to *NEx*. During CON exercise, lipolysis was higher in *Ex* compared to *NEx* without differences in plasma catecholamines, atrial natriuretic peptide (ANP) and insulin concentrations. Additionally, FOx was higher in *Ex* compared to *NEx* sessions. During CHO exercise, lipolysis did not increased but remained higher in *Ex* compared to *NEx* sessions. Plasma catecholamines and ANP were not different compared to CON. Plasma insulin level increased during CHO exercise while remaining lower in *Ex* compared to *NEx*. FOx was blunted during CHO exercise in both session but still remained higher in *Ex* compared to *NEx*. At rest, and during CON and CHO exercises, higher plasma fatty acid concentrations were recorded with higher lipid mobilization in *Ex* compared to *NEx*.
Conclusion: In conclusion, lipid mobilization and plasma fatty acid availability are strong determinants of FOx in endurance trained men.

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One year endurance training: effects on lung function and airway inflammation

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Introduction: This study was designed to assess airway changes occurring during the course of a sport season in endurance runners, and the possible relationship between induced sputum histamine concentration and inflammatory processes in the lung.

Methods: In 10 healthy endurance trained runners (mean age ± SD: 19 ± 3 yr), induced sputum (IS) samples were obtained at rest and 2 h after an exercise session at 80% of athlete's maximal aerobic speed. Respiratory function was analysed either after exercise, or at rest through spirometry. The tests were conducted on three different occasions: during basic endurance training and then during the pre-competition and competitive periods.
Results: Instead the absence of post-exercise respiratory symptoms or spirometric changes, airway cell counts change significantly. At rest, IS showed prevalence of macrophages (40%) over neutrophils (25.8%); after exercise, total cells increased significantly during the competitive period. Increased neutrophil counts were found in the two last periods of the sport season ($P < 0.05$). A significant increase in macrophage counts was also observed during the pre-competitive period. IS supernatant histamine concentration also increased in the competitive period ($P < 0.05$). However, no correlation was found between sputum histamine and sputum cell counts.
Conclusion: In non asthmatic endurance runners, a year of training increased markers of inflammation in the airways without symptoms or changes in pulmonary function. Because of the absence of significant correlation between sputum histamine and all airway cell types, our data do not provide a clear-cut role for histamine in the context of exercise induced airway inflammation. Physical exertion, causes significant stress to the respiratory system. Associated hyperventilation and airway exposure to contaminants of inhaled air could explain cellular changes.

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Effect of high-fat diet and physical training on carbohydrate metabolism in rat

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Introduction: Physical exercise affects many of both homeostatic mechanisms in which endocrine and metabolic systems are closely involved. There is much interest in the health advantages of exercise, but there is also interest in diet influences on exercise performance. The purpose of the present study was to determine the effects of high-fat diet (olive oil) and exercise training on plasma levels of insulin, glucose and muscle glycogen.
Methods: The study was carried out with four groups of male rats subjected for 4 weeks to a diet rich in olive oil and exercise training. The four groups were composed of: the normal diet control (NC; $n = 12$), the high-fat diet control (FC; $n = 12$), normal diet with exercise (NE; $n = 12$) and high-fat diet with exercise (FE; $n = 12$). Animals in high-fat diet groups were fed ad libitum diet containing 10% olive oil (weight/weight). After 4 weeks on the diet, as baseline period, animals were divided into sedentary and exercised groups, and diet was continued for an additional 4 weeks. The trained groups swam 1 hour/day 5 times weekly for 4 weeks. All groups were killed 24 hours after the last bout of exercise. Insulin concentrations, glucose levels and muscle glycogen were analysed. Analysis of variance was used to test differences among groups.
Results: Feeding rats with high-fat diet increased insulin levels ($P < 0.05$), glycaemia ($P < 0.001$), and muscle glycogen ($P < 0.001$).
Conclusion: Results from the present study demonstrated that high-fat diet has important effects in the carbohydrate metabolism when the diet was associated with training. These alterations are generally considered to favour endurance performance capacity.

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Effect of wingate test on concentration of superoxide dismutase in judo athletes plasma

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Introduction: Superoxide dismutase catalyse the dismutation of superoxide anion to molecular oxygen and hydrogen peroxide, and it is likely that they protect cells against toxicity of free radicals. The possibility that this could cause oxidative tissue damage including skeletal muscles is upheld by much experimental work. However, if the effect of an acute bout of exercice in animals has uniformly resulted in an increase in tissue lipid peroxidation, the data in human is not at all in agreement. A little is known about the effect of supramaximal exercise on superoxide dismutase in judoka athletes. The aim of this study was to determine the effect of wingate test on plasma superoxide dismutase.
Methods: Ten healthy male judoka athletes, aged 19–22 years, voluntered for this study underwent the wingate test. The exercise test, performed in the morning after an overnight fast, consisted of 30 seconds work periods on a monark bicycle ergometer at 120% VO2max. On the two days preceding the experiment athletes were requested not to take exercise. Heparinzed blood samples were collected by venepuncture before, at the start and end of the exercise session and then 5, 10 and 20 minutes later. Plasma was carefully aspired off, leaving the buffy coat and packed erythrocytes and then stored at –80°C for 2 weeks until the time of enzyme analysis. Measurements of immunoreactive superoxide dismutase were made by ELISA. Total antioxydative status was also determined.
Results: Results are shown in table 1. There were significant increase in plasma superoxyde dismutase activities as well as hematocrit level immediately after the exercise and until 10 minutes after exercise period without change in total oxidative status suggesting some effects of acute exercise on skeletal muscles.

	Control values	0 min**	5 min*	10 min	20 min
Superoxyde Dismutase: UI/mL	0.644 ± 0.03	0.788 ± 0.04	0.774 ± 0.08	0.741 ± 0.06	0.669 ± 0.07
Antioxydative Status: UI/mL	1.796 ± 0.01	1.770 ± 0.01	1.802 ± 0.05	1.803 ± 0.06	1.798 ± 0.01
Hematocrit: %)	43 ± 0.8	46.6 ± 1.1	44.2 ± 0.1	44.1 ± 0.8	44.2 ± 0.8

Conclusion: Brief supramaximal exercise induce transient increase in superoxide dismutase in judoka athletes that could protect skeletal muscle from oxidative stress.

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Swallowing sounds after partial or total laryngectomy

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Introduction: We demonstrated that three main sound components (SC) are included in the swallowing sound. The aim of this study was to evaluate the changes in SCs after total (TL) or partial supracricoid (SCL) laryngectomy. Patients with neck cancer were examined using our digital acoustic recording technique for swallowing before and after surgery.
Methods: Twenty patients were assigned to the SCL group (11) or the LT group (9). A microphone was put in place below the cricoid cartilage and connected to the computer acquisition card. Six recordings were realized before surgery and 6 months after. The COOLEDIT PRO software was used to the sound analysis. The mean number of SCs (N), the mean duration of total sound (SD), the mean duration of the three components (SC1D, SC2D, SC3D) and the percentage of presence for SC1, SC2 and SC3 for all the recordings were calculated.
Results: SCL group. 53 recordings were realized before surgery, and 54 after. N before and N after were not different (2.4 vs. 2.5). The presence of SC1 was higher (86% vs. 66%) before surgery and the presence of SC3 was higher (83% vs. 41%) after surgery. SD was not significantly different before-after (701 vs. 622 msec, $P = 0.33$). SC1D was significantly enhanced (78 vs. 44 msec; $P = 0.022$) after surgery. The signal frequency was not significantly different (527 vs. 492 Hz). LT group. 50 recordings were realized before surgery, and 40 after (2 early dead patients). N before and N after were not different (2.15 vs. 1.88). SC1 and SC3 were lower after surgery, (SC1 54% vs. 74%; SC3 27% vs. 44%). SD after was reduced (296 vs. 562 msec; $P = 0.001$). SC1D, SC2D, SC3D and F were not significantly different after surgery ($P > 0.05$) i.e. 69 vs. 56 msec, 196 vs. 153 msec, 63 vs. 45 msec and 839 vs. 867 Hz respectively.
Conclusion: We demonstrated that after surgery, the percentage of presence of SC and their duration were modified. These variations were the result of the anatomical restructuration of the pharynx and the larynx in relation to partial and total laryngectomies.

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Effects of Artemisia herba-alba Asso. aerial parts on high-fat diet induced obesity in rats

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Introduction: Artemisia herba-alba Asso. (Compositae) has been widely used in North African folk medicine for the treatment of high blood pressure and gastrointestinal disorders. A few clinical studies have shown beneficial effects of this plant on diabetes mellitus.
In this study, we investigated Artemisia hole aerial parts bioactivity on obesity promoted in rats by high-fat diet.
Methods: Male Wistar rats (200–210 g) were divided into three dietary groups ($n = 8$ for each); the first group receives chow containing 15% animal fats. In addition, rats of the second group were fed with 4% (3.6 g/kg/day) safe dietary dose of Artemisia's aerial parts. The control group receives standard pellets. After 8 weeks, rats were sacrificed; results on body weight gain, liver, fat pad mass index were recorded.
Results: Plasma samples were collected to determinate glucose, urea, creatinine and lipids plasma levels. Dietary supplementation of Artemisia decreases significantly overweight induced by high-fat diet (48%; $P < 0.001$). Furthermore, significant reduction in abdominal and liver mass indexes, at once, were observed among treated rats with respective percentages of 34% and 10% ($P < 0.001$). Increased fasting blood glucose concentration, induced by high-fat diet, were significantly attenuated in Artemisia-fed rats (12%; $P < 0.05$). No significant effects were observed in triglycerides and total cholesterol levels although a minor increase in HDL fraction with Artemisia-fed rats (13%; $P < 0.05$). Otherwise, treatment reduces efficiently hypercreatinemiea induced by high-fat diet (23%; $P < 0.01$) but have no effect on urea level.
Conclusion: These findings suggest that Artemisia herba-alba could be used as a valuable nutritional supplement to improve some obesity related disorders.

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Lipemia, tissular lipids and pregnancy in Algerian domestic rabbit

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Introduction: Pregnancy consists in complexes physiological processes including immuological and hormonal events with metabolic impacts. Because the placentation in rabbit occurs at the same way as that in Human, the local domestic rabbit, characterized below is used as model to determine the influence of pregnancy on lipid metabolism.
Methods: Monitoring of biochemical plasmatic parameters (TG, cholesterol, lipoproteins), histochemical (Soudan black B) and biochemical (Folch's method) aorta studies drawn from normal and pregnant rabbits show many differences.
Results: A significant cholesterolemia fall is registered in the end of pregnancy whereas TG decrease during the gestation and rise before the parturition. A reduction of VLDL + LDL in pregnant is noted comparatively to control (31.53 ± 9.27 mg/dl vs. 63.6 ± 30.71 mg/dl).
Total aortic lipids content rise during pregnancy with essentially TG form, which presents an elevated concentration (0.43 ± 0.12 vs. 0.14 ± 0.07 mg/dl, $P < 0.01$). Soudan Black coloration show a meaningful aggregation of lipid rich cells to vascular endothelium, but few lipid reserves are observed between slices in the media. In the liver, TG concentration rises and cholesterol level decreases ($P < 0.01$).
Conclusion: It seems that pregnancy reduces lipid stocks in the local rabbit liver and rises lipids capture and storage in the aorta. Pregnant hyperlipidemia registered in Algerian domestic rabbit seems no pathogenic, this physiological state normalizes after the parturition.

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Effect of nitric oxide inhibition on glycogen phosphorylase and hexokinase activities in rat liver after water or food deprivation

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Introduction: Nitric Oxide is a short-lived metabolite with multiple biological activities. Among the many biological effects of nitric oxide is a modulation of multiple pathways in glucose metabolism. Indeed, it has been shown that in perfused livers from fed rats, either no effect, inhibition or stimulation of glycogenolysis have been observed, depending on the experimental conditions used. In the present investigation, the possible interference of nitric oxide in glucose metabolism was studied in three-day starved or dehydrated rats after administration of L-nitro-arginine methyl ester, a widely used nitric oxide synthase inhibitor (L-NAME).

Methods: Saline and L-NAME were administered by intra-peritoneal injection in the amount of 50 mg/kg body weight to rat submitted to food or water deprivation for three days and to control rats. At the end of the experiment, the liver was removed. The glycogen rate was measured by gravimetric method. The activities of glycogen phosphorylase and hexokinase were analysed in the homogenate. The methodology employed was a non denaturing electrophoresis followed by activity-staining (native PAGE).

Results: Our results showed that in control rats L-NAME administration increased Glycogen phosphorylase activity by 36% and decreased hexokinase activity by 0.84%, but in dehydrated and starved rats L-NAME injection decreased glycogen phosphorylase activity respectively by 21.48% and 15.70% and increased hexokinase activity respectively by 7.24% and 10.78%, compared to saline injected rats. Liver glycogen rates were not significantly affected by L-NAME administration. In addition, we found that in saline treated animals, three days of water or food deprivation increased glycogen phosphorylase activity respectively by 47% and 63% and decreased hexokinase activity respectively by 29.34% and 25.16%.

Conclusion: Thus, we conclude that nitric oxide may be involved in the regulation of glucose metabolism by modulating the activities of hepatic enzymes of glycogenolysis and glycolysis according to physiological conditions.

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Cortical plasticity of swallowing oral muscle induced by ventilation and swallowing tasks

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Introduction: In stroke patients swallowing rehabilitation is the main treatment of swallowing disorders. Nevertheless, it has never been demonstrated if such rehabilitation could enhance plasticity of swallowing muscle cortical areas. The aim of our study was, in healthy subjects, to appreciate changes in cortical representation and cortical excitability induced by ventilation or swallowing tasks.

Methods: In nine healthy right handle subjects (five females, age range 20–26 yrs), surface mylohyoid EMGs and pharyngeal pressure were recorded. Non focal transcranial stimulation (nfTMS) and focal stimulation (fTMS) of MH were performed, permitting to measure MHMEPs and modification in pharyngeal pressure. Thresholds were also measured during nfTMS and fTMS. During focal stimulation, cortical area was determined for the right MH muscle (number of point spaced by 2 cm able to product MHMEP). During nfTMS, MHMEP were realised during expiration, swallowing and sniff manoeuvre. This was performed initially and one week later to assess the reproducibility. Thereafter, subjects were asked to realise 10 minutes, everyday during one week, ventilation with glottic movements or swallowing tasks. Each subjects made the trials in a random order. After each week, the subjects were re-evaluated.

Results: Swallowing tasks increased the cortical representation of MH muscles, as ventilation did not [Swallowing trial: $n = 13 \pm 7$ points ($P = 0.05$), Ventilation trial: 11 ± 5 points (ns)]. Amplitudes of the MHMEPs and amplitudes of pharyngeal pressure induced by nfTMS increased after ventilation movements and not after swallowing tasks. They were highest during swallowing.

Conclusion: Swallowing task and ventilation movements modified cortical representation of swallowing oral muscles, the first one increased cortical representation and facilitation as the second one could modify long term plasticity.

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Activity of adiponectin, leptin and cytokines in diabetic mothers and their macrosomic offspring

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Introduction: Not much is known about the implication of adipokines and different cytokines in gestational diabetes mellitus (GDM) and macrosomia.

The purpose of this study was to assess the profile of these hormones and cytokines in macrosomic babies, born to gestational diabetic women.

A total of 59 women (age, 19–42 yr) suffering from GDM with their macrosomic babies (4.35–0.06 kg) and 60 healthy age-matched pregnant women and their newborns (3.22–0.08 kg) were selected.

Methods: Serum adipokines (adiponectin and leptin) were quantified using an obesity related multiple ELISA microarray kit. The concentrations of serum cytokines were determined by ELISA.

Results: Serum adiponectin levels were decreased, whereas the concentrations of leptin, inflammatory cytokines, such as IL-6 and TNFα, were significantly increased in gestational diabetic mothers compared with control women.

The levels of these adipocytokines were diminished in macrosomic babies in comparison with their agematched control newborns.

Serum concentrations of T helper type 1 (Th1) cytokines (IL-2 and interferon γ) were decreased, whereas IL-10 levels were significantly enhanced in gestational diabetic mothers compared with control women.

Macrosomic children exhibited high levels of Th1 cytokines and low levels of IL-10 compared with control infants.

Serum IL-4 levels were not altered between gestational diabetic mothers and control mothers or the macrosomic babies and newborn control babies.

Conclusion: GDM is linked to the down-regulation of adiponectin along with Th1 cytokines and up-regulation of leptin and inflammatory cytokines. Macrosomia was associated with the up-regulation of Th1 cytokines and the down-regulation of the obesity-related agents (IL-6 and TNFα, leptin, and adiponectin) (*J Clin Endocrinol Metab* 91: 4137–4143, 2006).

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Effect of subchronic exposure to organophosphorus compound on glucose metabolism in rats *in vivo*

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Introduction: The widespread use of pesticides in public health and agricultural programs has resulted in pollution of water, air, and food that including severe acute and chronic cases of human poisonings. In addition, the use of organophosphorus pesticides has increased considerably due to their low potency and durability as compared to organochlorine pesticides. Organophosphorus compounds are primarily recognized for their ability to induce toxicity in mammals through inhibition of acethylcholinesterase leading to accumulation of acetylcholine and subsequent activation of cholinergic muscarnic and nicotinic receptors. The aim of this study was the evaluation of the effects of a subchronic exposure to malathion, an organophosphorus insecticide, on hepatic enzymes of glycogenolysis and glycolysis in rats *in vivo*.

Methods: Malathion was administered intragastrically by stomach tube in the amount of 1 ml corn oil containing 100 mg/kg body weight daily for 32 days. At the end of the experiment, the liver was removed. The activities of glycogen phosphorylase and hexokinase were analysed in liver homogenates. The methodology employed was a non denaturing electrophoresis followed by activity-staining (native Plyacrylamide Gel Electrophoresis).

Results: Malathion decreases glycogen phosphorylase activity by 50% and increases hexokinase1 activity by 10%. In addition, an hepatomegaly was recorded with a rise in the hepatic glycogen rate in malathion treated rats. The storage of glycogen in liver may be due to a stimulation of insulin secretion after the inhibition of acethylcholinesterase activity in pancreatic beta cells by malathion. These findings were in favour of an activation of glycogen storage by malathion.

Conclusion: In conclusion, malathion disrupted the glucose metabolism and more attention is needed to limit the use of organophosphorus insecticides as much as possible and their entrance into the human food cycle.

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Oral stimulation and oral support increase feeding performance in premature infants: assessment by acoustic technique

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Introduction: The acoustic technique is non-invasive and atraumatic and can be used in premature neonates. The aim of this study was to compare the effects of oral stimulation (Stim) and oral support (Sup) on nutritive sucking.

Methods: Premature infants born between 29 and 34 weeks' GA were allocated to three experimental (Stim + Sup – Stim-Sup) and Control groups. A microphone was put in place on the neck and connected to a minidisk recorder. The swallowing activity recorded was transferred from the MD recorder to computer for quantitative analysis of feeding. Number of swallows (S) and swallow bursts (B) were quantified in the transition period. Isolated swallows and swallows in bursts i.e. Small bursts (<6 swallows), middle size bursts ($6 \leq$ swallows ≤ 9) and large bursts (swallows > 9) were quantified. Histograms were drawn with swallows in bursts for the four groups to reveal swallowing behaviour. Variables were analysed using repeated-measures analysis of variance with birth weight as covariate (ANCOVA).

Results: S and B were significantly increased for the three experimental groups compared to the Control group. Isolated swallows were predominant for the Control group compared to the three experimental groups. Middle size bursts were predominant for the Stim + Sup and Sup groups compared to the Stim and Control groups. Histograms were drawn and swallowing behaviours were clearly visualized on the histograms: bursts were more irregular for the Stim and Control groups than for the Stim + Sup and Sup groups.

Conclusion: We demonstrated that oral support enhances swallows and swallow bursts of middle size to facilitate the full oral feeding. Oral support stabilises the jaw, reinforces deglutition and organises ingestion with imposed rhythm to obtain full oral feeding. It can be considered as training for endurance and sucking-swallowing-breathing coordination. Individual histogram are thus able to identify the level of swallowing function in the neonate. Research is planned to provide better understanding of the mechanism involved in such oral support.

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Atrial natriuretic peptide increases lipid mobilization and utilization during head-down bed rest in man

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Introduction: Head-down bed rest (HDBR) increases plasma levels of atrial natriuretic peptide (ANP) and decreases norepinephrine levels. We have previously demonstrated that ANP promotes lipid mobilization and utilization when infused into lean healthy men at pharmacological doses. The purpose of the current study was to demonstrate that a physiological increase in ANP is able to induce lipid mobilization and oxidation.

Methods: Eight men where positioned for 4 hours in a sitting (control) or in a HDBR position. Indices of lipid mobilization and hormonal changes were measured in plasma. Extracellular glycerol, an index of lipolysis was determined in subcutaneous adipose tissue (SCAT) using a microdialysis technique.

Results: A twofold increase in plasma ANP concentration was observed after 60 min of HDBR and a plateau was maintained thereafter. Plasma norepinephrine decreased by 30–40% during HDBR, while plasma insulin and glucose levels did not

change. The level of plasma NEFA was higher during HDBR. SCAT lipolysis, as reflected by interstitial glycerol, as well as interstitial cGMP, the second messenger of the ANP-pathway, increased during HDBR. This was associated with an increase in blood flow observed throughout the HDBR. Significant changes in the respiratory exchange ratio, and percentage use of lipid and carbohydrate were seen only after 3 h of HDBR. Thus, the proportion of lipid oxidized increased by 40% after 3 h of HDBR.
Conclusion: The rise in plasma ANP during HDBR was associated with increased lipolysis in SCAT and whole-body lipid oxidation. In this physiological setting, our study demonstrates that ANP stimulates lipid mobilization and oxidation in human.

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Abstract withdrawn

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Short-term exposition to saturated fatty acids inhibits adrenergic alpha2-antilipolytic effect in human adipose tissue
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Introduction: The aim of the study was to investigate *in vitro* and *in vivo* the acute effect of saturated fatty acids (FA) on the regulation of adrenergic lipolysis in human adipose tissue.
Methods: We tested first *in vitro* on isolated fat cells, the effect a 2-hours treatment with saturated FA. Then, *in vivo*, eight healthy lean and six obese males' subjects performed a 45 min exercise bout at 50% of their maximal oxygen uptake either after an overnight fast or 3 hours after ingestion of a high fat meal (HFM) (95% fat and 5% carbohydrates). Subcutaneous adipose tissue lipolysis was measured by microdialysis in the presence or absence of an alpha-blocker (phentolamine). Dialysate glycerol concentration was taken as an index of lipolysis.
Results: *In vitro,* the results show that acute treatment of fat cells with 200 μM of saturated FA enhances the lipolytic effect of epinephrine by suppression of the antilipolytic alpha$_2$-adrenergic effect. *In vivo,* HFM increased plasma non-esterified fatty acid level by 2-fold in lean and obese subjects. In both group, HFM ingestion do not alter glycerol kinetic and hormonal response to exercise. In fasting condition, the alpha$_2$-adrenergic antilipolytic effect was more pronounced in obese than in lean subjects. HFM totally suppressed the alpha$_2$-adrenergic antilipolytic effect during exercise in lean and obese subjects.
Conclusion: The present result demonstrates that saturated FA per se, *in vitro* as well as *in vivo*, suppresses alpha$_2$-adrenergic mediated antilipolysis in adipose tissue. This is another mechanism whereby fat-rich food may contribute to dysregulation of lipid metabolism.

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Determination of caloric contribution and study of deviation of the food behavior in 300 teenagers high school students
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Introduction: It is admitted that the period of adolescence is often the most period of fast growth. This period is often accompanied, by important biological, psychological and emotional evolutions. Thus, the food behavior could be disrupted considerably and some progressive deviations of the food behavior can be observed in the teenagers if they were not sensitized sufficiently. These deviations can are at the origin of various pathological states (obesity, diabetes, anorexia, …). In order to be able to determine these deviations in the food behaviour and their consequences on the health of the teenagers, we achieved an investigation among teenagers high school students.
Methods: Three hundred students aged of 15–20 years and coming from different zones (farming zone, urban zone and suburban zone) has been investigated. The investigation contains some information on the food contributions of these teenagers, their habits and their behavior opposite to the products light, omega 3 and omega 6. These products, benefitting from a media advertisement, invade day after day our daily life. Our study based on an investigation, concerning the anthropometric measures, the determination of the food contributions of the last 24 hours, the food behavior and the food habits.
Results: The analysis of our data allowed us to demonstrate that these teenagers are not sensitized sufficiently to be able to follow a correct food behavior. Indeed these teenagers showed a deviated food behaviour. Besides the determination of the caloric contributions of food showed that these contributions are disrupted in these teenagers. Our results showed a deficiency in iron, zinc, magnesium and in calcium for nearly the totality of these teenagers. On the other hand, we noted that several teenagers want to lose the weight, without knowing the physical and psychological risks that a non adapted slimming regime can entail. Other teenagers want to gain the weight, without knowing the risks on the health that a state of overweight can generate.
Conclusion: According to these data, caloric contributions of food were disrupted in these teenagers. In addition, it seems that most teenagers don't know again the true interest of these new products proposed by the medias. These last expose these products, talking always of their beneficial effects, but without evoking the inherent risks to their consumption.

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Behavioural disruption of human swallowing reaction times following a 1 Hz repetitive transcranial magnetic stimulation induced virtual lesion of swallowing motor cortex
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Introduction: 1 Hz repetitive transcranial magnetic stimulation (rTMS) can be used to inhibit cortex to induce virtual lesions of targeted brain regions. We applied a 1 Hz virtual lesion to healthy human swallowing motor cortex to establish whether such inhibition might induce behavioural changes to swallowing.
Methods: Eight healthy subjects (3 F; range 26–47 years) were recruited. Single pulse TMS (sTMS) was applied to swallowing motor cortex (SMC) to elicit pharyngeal motor evoked potentials (PMEPs) recorded via a swallowed intraluminal catheter. The hemisphere evoking the largest PMEPs was termed the dominant SMC (DSMC). Following baseline sTMS recordings of both ipsilateral DSMC and contralateral non-dominant SMC (NDSMC), a baseline swallowing reaction time task was performed comprising three cued tasks: normal, fast and challenged swallows (within a 150 ms time window). Thereafter, 10 min of rTMS (real or sham, randomised to separate days) was applied to the DSMC. STMS was then reapplied, immediately, 30 and 60 min after rTMS (or sham), in conjunction with the swallowing tasks. Baseline vs. Intervention data (real and sham) were then analysed with ANOVA.
Results: PMEP amplitudes from the DSMC decreased by 21%, after real but not sham rTMS, $P < 0.01$, returning to baseline by 60 min. After real rTMS, swallow reaction times were reduced by 11% for both normal and fast swallows compared to baseline, $P < 0.001$ not seen with sham rTMS. Challenged swallows failed to improve with real rTMS, but showed an expected rise in successful hits with sham ($P < 0.05$).
Conclusion: Our data show a clear relationship between the SMC, excitability and swallowing performance and provides a human model for cortical dysfunction after a virtual brain lesion.

