1 Allosthesia in visual and auditory sensations from environmental signals

Introduction: ‘Allosthesia’ describes the fact that sensory stimuli can arouse pleasant or unpleasant sensations according to the internal state of a person. In the present study we investigated if the hedonity 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 y) took part in 5 experimental sessions. During all sessions, participants stayed in an 11.6 m² quiet black room, with white walls, and without decoration or furnishing. Sessions differed according to 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 analog scales as, ‘Tiredness’, ‘Hedonics 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 > 0.05) with the state of tiredness.

Conclusion: Allosthesia occurred in visual and auditory sensations that originated from the environment, and motivated behaviours that were not related to the environment.

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

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 epilepsy (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-electrocorticography, 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 the same session using a spectrum analysis.

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; r = 0.541). This correlation was observed when considering either mesial ROIs (P = 0.002) or neocortical ROIs (P = 0.05).

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

Introduction: Serotoninergic 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. Eight years ago significant progress in the pharmacology of neurologic drugs side effects such as somnolence, vigilance decrease, addition, and enzympic induction remain a concern. The aim of the present investigation is to find a new neuroleptic agent with fewer drugs 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 brain was dissected in liquid nitrogen, frozen, 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-+(6-Methoxy-2,3-dihydro-[1,4]dioxino [2,3-b] pyridin-3-yl methyl) amine] butyryl] -8-anu-spiro-[4,5] decane-7,9-dione from a pyridine derivative. 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^-7M). By behavioral experiments, 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 decreased the animal aggressiveness when either 5 mg/kg or when 30 mg/kg were used. Neurochemical studies showed that tryptophan concentration increased 20 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 neurologic activities in mice probably by interacting with indoleaminergic system.

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

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 family, and it was found that TREK-1 channel and its splice variants are expressed at the lowest level. It is present in the peripheral sensory system, in particular in small dorsal root ganglion (DRG) neurons that are associated with noxious perception. This channel also has a high temperature sensitivity as it is activated at 31°C and 40°C, making it ideal for detection of cold and/or heat in addition to mechanical stimuli. The objective of this work is (1) to examine the exact localization of channels TREK-1 in different type 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 anoxia warm and painful heat. Knockout animals also display the increased thermal and mechanical hypalgesia in conditions of inflammation proving the important role for TREK-1 in polymodal pain perception. They display a largely decreased response to hypoosmotic 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 serotonergic receptors on scopolamine-induced memory deficits in mice

Introduction: Involvement of 5-HT6 receptors (5-HT6R) in learning and memory 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 consolidation 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: The results demonstrated 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 (1 mg/kg) 30 min after the training. The 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 completely.

Conclusion: We thus hypothesize that mouse 5-HT6R are, as in the rat, implicated in the regulation of the cholinergic neurotransmission and that such a localization 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 
I. Raad, K. Othman1, S. Asaichat1, Y. Rentrozzi1, *Alger – Algeria

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: Rats were administered 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 regularised. 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 histological 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 parameters. Colometric analysis of extracellular matrix proteins report that methionine intake increases collagenose in pancreas and a non collagenous proteins in adrenal. Hence, methionine seem decrease non collagenous proteins in pancreas. Any significant effect is enregistered in kidney.

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

7  DHA modulates Ca2+ signaling through the phosphorylation of tyrosine kinases in human Jurkat-T cells 
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Introduction: The goal of our study is to focus on the role of PTK in the DHA modulation of calcium signaling in Jurkat T cells. Pre-incubation of Jurkat T cells with p59fynT, a selective inhibitor of PTK, significantly potentiated DHA-induced rise in intracellular Ca2+

Methods: The concentration in free intracellular Ca2+

Results: DHA induced dose-dependent increases in [Ca2+]i which were concomitant with an increase of tyrosine phosphorylation of PTK. The increase in [Ca2+]i was significantly lower in Jurkat T cells pre-incubated with p59fynT, a specific inhibitor of PTK, as well as with SU6656, a specific inhibitor of p59fynT. In addition, the p59fynT inhibitor significantly potentiated DHA-induced rise in [Ca2+]i.

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

8  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 causal of the onset of obesity, 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 we, a four-week HFD feeding, which induced obesity and diabetes, chronically increased LPS concentration 2-3 times, a threshold that we defined as "metabolic endotoxemia".

Results: When oral administration of LPS was performed, the endotoxemia was not modified, whereas the endotoxemia was modified in mice that were challenged with LPS by immunoprecipitation experiments showing that DHA induced p59fynT phosphorylation, which was found to be potentiated by either P2 or SU6656.

Conclusion: This study shows that dietary DHA can act through the activation of CRAC channels and p59fynT activation in Jurkat T cell line.

9  Regulatory T cell functions are regulated by a dietary fatty acid, docosahexaenoic acid (DHA), in wild type and PPARγ-null mice 
A. Yessoufou1, A. Pleb2, A. Hichami2, K. Moutariou3, NA Khan4, *Dijon – France / College of Medicine – King Faisal University – Saudi Arabia

Introduction: CD4+CD25+ regulatory T (T-reg) cells are essential for the induction and maintenance of immunological self-tolerance as well as transplantaion tolerance and several studies 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 responses.

Methods: Responders 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 significantly increased the 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 (PPARγ)-null mice than in wild type (WT) mice. However, the index on anti-CD3-stimulated T cell proliferation was higher in WT mice (3 fold) than in PPARγ-null mice (2.1 fold). In addition, the inhibitory action of T-reg cells was also weakened when CD4+ T responder were pre-incubated with DHA. DHA also increased the expression of a wide range of genes including forkhead/winged helix transcription factor (FOXP3), cytokotic T lymphocyte-associated antigen (CTLA)-4 and transforming growth factor (TGF)-β in T-reg cells from PPARγ-null mice, whereas it decreased their expression in T-reg cells from WT mice.

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

10  Morphological regionalization and multidimensional analysis effects of castration and efferent duct ligation on the proximal epididymal segment of sand rat (Psammomys obesus) 
R. Menad1, L. Lakabi1, A. Boubeker1, A. Messi2, G. Terngorn

Introduction: In the mammals, the epididymis duct is not a simple channel of transport but it also plays a role in the storage of a true reservoir by a true blockage and by a true storage. 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 Beni Abbès (Algeria). In the autumn (breeding season), adult males castrated and 30 days later, some were killed, whilst others were injected with testosterone enanthate 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: Comparison between regions were calculated using analysis the variance (ANOVA). The repercussion study on the cellular morphology has necessarily the principal component analysis (ACP).

Results: 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. The correlation between these results makes it possible to classify these two parameters as explanatory factors for the exploration of the androgens and/ or the testicular factors deprivation effects on cells morphology.
Total RNA from seminal vesicles was translated in a cell-free system derived from rabbit reticulocyte lysate and 35S methionine. Two major bands of Mr 14 400 and 21 000 were visualized by denaturing gel electrophoresis. Total RNA from seminal vesicles was translated in a cell-free system derived from rabbit reticulocyte lysate and 35S methionine. Two major bands of Mr 14 400 and 21 000 were visualized by denaturing gel electrophoresis.

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 

Conclusion: In this work, we demonstrated that 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 vivo cell-free translation experiments. Last, we characterized some important nuclear genes targeted by this pathway.

Methods: To assess 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.

Conclusion: We recently reported [2] that POF programs VAT micro-inflammation and perinatal programming of obesity and the metabolic syndrome. Methods: Postnatal overfeeding (POF) was induced in the rat by a reduction of the daily calorie intake by 25% at 7 days of postnatal age. We used an in vivo cell-free translation system to study the mitochondrial import of these receptors. Results: The purpose of this study was to examine the sexual maturation in Tunisian children.

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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.
17 Central role of NO in ventilatory acclimatization to hypoxia in a model of anemic transgenic mice
Introduction: Polycythemia and increase in ventilation are considered as important factors in hypoxic ventilatory response. Despite low oxygen carrying capacity, it has been recently shown that anemic transgenic mice (Epo-TAgh mice) adapt to chronic hypoxia partly through an increase in ventilatory acclimatization (VAH), which is accompanied by an increase in normoxic ventilation in Epo-TAgh mice; 2) in ventilatory acclimatization to hypoxia in both Wild Type and Epo-TAgh mice. Methods: For this study, twenty male anemic SV40 T antigen (Epo-TAgh) and twenty four male wild-type (C57Bl6/CBA, WT) mice, 8 weeks-old, were divided into four groups: a) Normoxic Epo-TAgh (Nx Epo-TAgh; n = 10); b) Normoxic Wild Type (Nx WT; n = 12); c) Hypoxic Epo-TAgh (Hx Epo-TAgh; 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 acclimatization to hypoxia are enhanced in Epo-TAgh mice. In normoxia, NOx was lower only in plasma (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 M CCh stimulation, and there was no additional delayed contraction. FFmax was in T: 35.27 ± 6.50 % ACh; in EPB: 28.61 ± 4.2 % ACh; in IPB: 24.78 ± 5.2 % ACh. Incubation with Y27632 did not modify the baseline tension. In response to 10 μM CCh, Y27632 abolished almost completely the contractile response (fFmax, time to half-fFmax, (Tf50), and Hill coefficient (n). The amplitude of the fast early phase of the contraction, influencing force amplitude, correlated well with elevated levels of RAGE, a marker of alveolar epithelial injury (< 0.005; see Figure) and in the perfusate 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 plasma. Conclusions: Our results indicated that lungs with impaired alveolar epithelial fluid clearance in a novel isolated perfused human lung preparation using lungs rejected for transplantation.

19 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 (AE(II)) 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. Methods: Transgenic mice harboring the Liddle R566-stop gain-of-function mutation (beta-ENaC C-terminus deletion leading to increased ENaC endocytosis and increased channel activity). Gi was studied. ENaC subunit expression was assessed by real time RT-PCR and Western blotting in wild type (+/+), heterozygous (PT), and L/L mice. Results: Plasma and alveolar fluid sodium concentration, and Na+ transport were significantly higher in L/L mice compared with +/+ mice. Conclusion: Our data demonstrate that increased amiloride-sensitive Na+ transport occurs in L/L mice. Alveolar fluid accumulation was associated with impaired alveolar epithelial Na+ reabsorption at baseline, and develop less severe pulmonary edema when exposed to an experimental model of volume overload, suggesting that increased Na+ reabsorption protects from alveolar edema in adult lung.

20 Impaired alveolar fluid clearance in isolated perfused human lungs correlates well with elevated levels of RAGE, a marker of alveolar epithelial type 1 cell injury
R Briois1, J Frank2, M Matthay3* Grenoble – France * San Francisco – Etats-Unis
Introduction: Recent studies have established that alveolar epithelial type 1 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 1 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.
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Compliance with prescription mandated in children after emergency department (ED) visit
H Chapuy*, G Cheron1, S Faesch1, A Gary1, JM Trehalley* - Paris - France

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 education after an 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.

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 pain 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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The therapeutic guidelines database: an original new tool

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 weighted with grades of recommendation corresponding to scientific levels of evidence, as defined by the HAS (high authority 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 French, medical terms into MeSH (medical subject 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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Key role of bone marrow estrogen receptor alpha and FGF2 on the effect of estradiol on reendothelialization

Introduction: 17ß-Estradiol (E2) accelerates reendothelialization, but the cellular and molecular events are poorly documented. We aimed the use 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 RedIl 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 reendothelialization 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
27 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 diabetes. 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 coronaryography 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 coldpressor test (CPT) (n = 25).

Results: Patients were classified in three groups: group 1: no SMI (n = 26); group 2: SMI no coronary artery stenosis (n = 17) and CEF 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 were significantly different (Diameter change: slope = 0.09 ± 0.06 vs. 0.08 ± 0.04 vs. 0.04 ± 0.01 for the brachial artery diameter and the LV relaxation index respectively, P < 0.05). There was a correlation between the changes in brachial artery diameter and the LV relaxation index (E/A ratio = 0.52, 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. Significant coronary artery stenosis and endothelial dysfunction is associated with an altered LV relaxation.

28 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 insulin resistance, increased cardiovascular and pulmonary vascular diseases. Epidemiological studies report an inverse association between dietary flavonoid consumption and mortality from cardiovascular diseases. The aim of this work was to study if dietary flavonoids of red wine (polyphenols) extract, Provinols™ in an experimental model of obesity, the Zucker fatty rats (ZFR).

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 by measuring the density, of intimal GFP-expressing cells 3 days post-injury. These experiments revealed that E2 increased the number, but not the density, of intimal GFP-expressing cells 3 days post-injury.

Results: Provinols™ increased NO production, reducing both oxidative stress and inflammation and mortality from cardiovascular diseases. The aim of this work was to assess the density, of intimal GFP-expressing cells 3 days post-injury. These experiments revealed that E2 increased the number, but not the density, of intimal GFP-expressing cells 3 days post-injury.

Conclusion: 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.
32 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-Follchet. The French Technical Committee on Immunization has proposed a vaccination campaign targeting children and young adults from 1 to 19 years old, living or studying in Seine-Maritime, with the use of a Norwegian meningococcal conjugate vaccine (MenBvac®) from 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 the department. A previous study has shown that the proportion of vaccine doses available for the vaccine of young children, an ‘active’ pharmacovigilance follow-up was set up by the AFSAPS. The objective of this study was to evaluate the nature, seriousness and incidence of adverse effects (AE) occurring within the 15 days following immunization.

Methods: The drug regulatory authority (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 administrator and/or the vaccine recipients/parents. The campaign began on June 12, 2006, targeting children from 1 to 19 years old living in the 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 (300) and a 2nd dose (62) 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%) 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%) consulted a GP not a child (1%). The most frequent AE in these first 5 years of age were fever (29%) and local effects, previously reported during clinical trials carried out by the NIPH with MenBvac® (1) administered in the same age group.

Conclusion: Disproportionality in spontaneous reporting databases increases after a safety alert, because of increased reporting of the event of interest, including events occurring after the alert. This may lead to an under-reporting of the event with other drugs or even three tuberculosises with other drugs after the alert vs. One before, or reporting of rhabdomyolysis with statins other than cerivastatin. 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.

Introduction: Exceptional heat waves have occurred in France during summers 2003 and 2006. Among the different risk factors of hospi-mortality during these periods, drug treatments may be one of the main 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 during both heat waves with 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 2003 reported to the French Network of Pharmacovigilance Centres and recorded in the French Pharmacovigilance Databse 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 (IHW2) and likely (IHW1). Data of 2006 were compared to the data observed in 2003.

Results: The total number of ‘serious’ ADRs registered 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 (26 IHW1, 29 IHW2 and 14 IHW3) with a maximal peak between the 10th and the 29th July. The most frequently ADRs (dehydratation, disturbance, neuroepithelial (confusion, sleepness), renal (acute renal insufficiency), general with hyperthermia. Drugs more frequently involved were diuretics, angiotensin converting enzyme inhibitors, antiparkinsonians, antifungal, antidepressant (mainly serotonin reuptake inhibitors), neuroleptics, digoxin and oral hypoglycemics.

Two main differences were found with 2003 heat wave: less antidepressant and antiepileptic, phosphorgical drugs were concerned and more antiparkinsonians, antiepileptic, hypoglycemic and anti infectious drugs were involved for a similar total number of reported ADRs linked to heat wave (65 in 2003, 285 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, while the study of the 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 antiparkinsonians, antiepileptic, hypoglycemic and anti infectious drugs may identify patients at risk, with other diseases.

39 Contrat de bon usage and pharmacovigilance H Hantz#, D Carthant Kowalski#, C Riche#* "Breiz – France".

Introduction: A French decree about the good medical practice concerning drug use was signed in 2001. As part of a local PV contrat de bon usage has been published in 2003 to help hospitals to describe the transfer of nelfinavir and its active metabolite M8 from maternal to fetal compartments. The main objective of this study was to describe the transfer of nelfinavir and its active metabolite M8 from maternal to amniotic fluid.

Methods: Nelfinavir and M8 concentrations in maternal plasma, umbilical plasma and amniotic fluid were 0.36 and 0.49 h^-1, to-amniotic fluid rate constants were 0.23 and 0.59 h^-1, formation clearance 1.17 L/h, formation and elimination half lifes were 2.07 and 2.51 h respectively.

Results: Despite some case reports of clinical suspicions, amoxicillin plus clavulanic acid is known to have no effect on the pharmacodynamic activity of acenocoumarol, as assessed by prothrombin ratio.

40 Nelfinavir-M8 pharmacokinetic modeling of placental transfer, a population study: Miremont-Salame#, A Abouelfath#, D Hirt#, S Urief#, V Julien#, G Firtion#, H Chappuy#, E Rey#, G Pons#, L Mandelbrot#, JM Treluyer"Paris – France; Colombes – France"

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 healthy volunteers were added to the database. Data were analysed with NONMEM software. Covariates were tested on PK and PD parameters including antibiotic treatment.

Conclusion: Despite some case reports of clinical suspicions, amoxicillin plus clavulanic acid has no effect on the pharmacodynamic activity of acenocoumarol, as assessed by prothrombin ratio.
41 APOMYGRE- a multicenter trial which validates myophenolone mofetil therapeutic drug monitoring in de novo kidney transplant recipients P Marguet\textsuperscript{a}, A Rousseau\textsuperscript{a}, J Debordo\textsuperscript{a}, G Hoitney\textsuperscript{a}, F Compagnon\textsuperscript{b}, L Hary\textsuperscript{c}, A Leichmann\textsuperscript{c}, A Taurand\textsuperscript{c}, D Debruyne\textsuperscript{c}, S Safvii\textsuperscript{a}, E Jacquet-Aigrain\textsuperscript{a}, Y Le Meur\textsuperscript{b}, lmage\textsuperscript{b} – France, Reims – France, Rouay – France, Aimsius – France, Strasbourg – France, Angers – France, Caen – France, Toulouse – France, Paris – France.

Introduction: Methods: Results: Conclusion: MMF to reach the predefined target. At M6, MPA AUCs and MMF daily dose were obtained with the FOCE method. By contrast, the empirical SE for the FO method was 19 vs. 31, n = 0.01. Patient and graft survival, as well as the incidence of adverse events were comparable in the two groups. Interestingly, biopsy proven acute rejection was 2.19 vs. 3.16 h.mg/L. Plasma MPA was determined using HPLC UV in all centers and MPA AUCs 0-12 h was calculated using a Bayesian estimator and a 1-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 PD group).

A total of 130 patients (65 in each group) could be evaluated. The AUC\textsubscript{0-12 h} was significantly higher in the CC group on day 14, M1 and M3 (34 vs. 27, 45 vs. 34 and 57 h.mg/L; P < 0.001). Due to an increased 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 compared to the PD group at M6, M12 and M24, respectively, achieving 2% vs. 12% (P < 0.001), 8% vs. 19% (P < 0.001), and 15% vs. 29% (P < 0.001) at M6, M12 and M24, respectively.

Conclusion: MTF UDM 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 hosphor-kinase (CPK); a myogenic electromyogram; muscle biopsy abnormalities diagnostic for or evoking a muscle disease; muscle degeneration proved by CT: creatine kinase > 340 h.mg/L or magnetic resonance imaging. In our region with that of controls selected through the Midi-Pyrénées Health Authority, the incidence of MMF discontinuation was significantly lower in the CC group (P < 0.01) at M6, M12 and M24. Biomarker for rejection was MPA AUC 12 h and MPA exposure in the whole group. The incidence of MMF discontinuation was significantly lower at M6, M12 and M24 in the CC group (P < 0.01) compared to the PD group.

Conclusion: 3 years 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 fibrates, P > 0.30). The estimated odds ratio of exposure to statins in patients to patients was 2.19 vs. 3.16 h.mg/L.

Conclusion: Patients developing chronic muscle disease 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.

44 Is it a link between nonsteroidal antiinflammatory drugs and severity of bacterial infections: a case control study? A Jouvenx\textsuperscript{a}, AP Jorville-Béra\textsuperscript{b}, H Bagheri\textsuperscript{a}, E Autret-Leca\textsuperscript{a} – Tours – France.

Introduction: Methods: Results: Conclusion: Several case reports suggest that lipid-lowering drugs, especially statins, could induce or favour the clinical expression of muscle diseases. However, a recent review of epidemiological studies of statins and AEs of the liver, the results of the present study were 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.

Introduction: Methods: Results: Conclusion: of death, graft loss, acute rejection episodes, MMF discontinuation) in the CC group

Methods: A total of 137 kidney transplant recipients were included. They were received a classical immunosuppressive regimen combining basiliximab, CSA, MMF (2 g/day until M6) and steroids (80 mg/day). The frequency of patients treated for 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\textsubscript{0-12 h}) of 200 h.mg/L. Plasma MPA was determined using HPLC UV in all centers and MPA AUCs 0-12 h was calculated using a Bayesian estimator and a 1-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 PD group).

A total of 130 patients (65 in each group) could be evaluated. The AUC\textsubscript{0-12 h} was significantly higher in the CC group on day 14, M1 and M3 (34 vs. 27, 45 vs. 34 and 57 h.mg/L; P < 0.001). Due to an increased 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 compared to the PD group at M6, M12 and M24, respectively, achieving 2% vs. 12% (P < 0.001), 8% vs. 19% (P < 0.001), and 15% vs. 29% (P < 0.001) at M6, M12 and M24, respectively.

Conclusion: MTF UDM 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 hosphor-kinase side effects.

82 Population design evaluation and optimisation for multiple responses models: application to the pharmacokinetics of AZT and AZT-TP C Bazzi\textsuperscript{a}, S Retout\textsuperscript{a}, F Mentre\textsuperscript{a} – Paris – France.

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 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 jointly fitting several 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, we designed 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 design in the context of multiple responses models based on the maximum information matrix. We then illustrate this approach for design evaluation and optimisation of prospective studies including the joint population PK modelling of multiple antidiabetic treatment (AMI) according to use of sulfonylureas (SU) as chronic pre-admission antidiabetic treatment.

Methods: 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 including coxibs and aspirin (>350 mg) was considered if by general route, whatever duration of exposure and if NSAIDs treatment was acute or chronic. Each case was matched to one control by age, site of infection and presence of diabetes. 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 of exposure and if NSAIDs treatment was acute or chronic. However in cases, the median delay before effective antibiotic therapy was twice more in those exposed to NSAIDs than in non-exposed cases (6 days [CI 2–3 days] vs. 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 antibiotic therapy 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 antibiotic therapy suggesting they mask the symptoms. Further studies are required to confirm that NSAIDs delay antibiotic therapy.
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 in osteoarthritic patients. Finally, characteristics of analgesic drug use were closely related to neuropathic pain suggesting that neuropathic pain could be predominant in PD patients.

48 Adrenal insufficiencies associated with inhaled corticosteroids: an under recognised event
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Introduction: Owing to the high 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 (>500 μg/day in children or >1000 μg/day in adults). The present study aimed 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 13 with AI and Cushing’s syndrome. Biological data confirming the diagnosis were provided in 12 cases (12 children and 20 adults). ICS used were fluticasone (n = 19), budesonide (n = 10) and beclomethasone (n = 5). 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 confirmed by 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-equivalent or more in children and 1000 μg/day in adults should be avoided. When a high dose of ICS is clinically required, an adrenal risk should be considered.

49 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 analyse the relationship between social and leisure activities and the use of psychoactive drugs among elderly people living at home.

Methods: We studied 5008 community-dwelling elderly persons aged 65 years or older 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, recreational activities such as TV watching). In addition, we studied 500 cases of psychotropic drug use reported taking at least one psychotropic drug during the month preceding the interview. In addition, an association between the use of psychoactive drugs and the kind of analgesic drugs. Their global consumption of analgesic drugs was not different in PD patients than in general population (61% vs. 53%) and to use chronically opioids, paracetamol, antiepileptics and antipsychotics.

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 in osteoarthritic patients. Finally, characteristics of analgesic drug use were closely related to neuropathic pain suggesting that neuropathic pain could be predominant in PD patients.
Methods: We extracted all high dosage buprenorphine deliveries reimbursed by the General Health Fund during eight periods (i.e., second trimester: years 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: (a) the incidence of requests (percentage of total delivered quantity delivered by doctor shopping) and (b) doctor shopping quantities (number of DDD obtained by doctor shopping) given in thousands of DDD (KDDD). We used delivery data to assess the incidence of patients on short and long-term treatment for nicotine dependence and the risk of non-compliance among exposed patients.

Conclusion: More research is needed to better understand the relationship between doctor shopping and high dosage buprenorphine deliveries. In the absence of centralization of all the exposed cases.

53 Male hypofertility and drug exposure: an analysis of observations recorded by the Pharmaco vigilance 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 Pharmaco vigilance database.

Methods: This study was based on spontaneous reports of adverse drug reactions submitted to French Pharmaco vigilance 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.

Results: A total of 66 cases of male fertility impairments were spontaneously reported from 1985 to March 2005, of which 51 reports were linked to drugs. The most reported drugs were listed according to the ATC classification (Anatomical Therapeutical and Chemical).

Conclusion: The study shows that neurological and antiinflammatory Drugs, known to impair 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.

54 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 unit over the last 10 years. For each report, demographic data, 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) use of a benzodiazepine in maternal use for psychiatric disorders.

Results: Data were available on 1108 neonates born to 1106 women (2 twin pregnancies) of whom 67% ingested one benzodiazepine during the whole pregnancy, 32% were exposed between 17 and 28 weeks, and 18% were exposed between 29 and 36 weeks. Twenty-one per cent of the neonates were prematurity with 9 small for gestational age. Twenty one neonates (19.4%, 95% CI: 12.5–28.2) experienced neonatal symptoms compatible with the response of a newborn to 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) use of a benzodiazepine in maternal use for psychiatric disorders.

Conclusion: The study shows that neurological and antiinflammatory Drugs, known to impair 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.
hepatic adverse events, it remains unclear whether or not hepatic adverse drug reactions are a cause or are related to the type of events. 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: The aim of our study was to describe the type and the delay of onset of the adverse events. We analysed the occurrence of the adverse events in 31 diabetic patients who were hospitalized for heart failure, oedema or and renal failure. 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 sulfonylureas 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 significant). Moreover, the results of the multiple logistic regression models taking into account potential confounding factors showed that thiazolidinediones exposing patients, isolated oedema, heart failure and oedema, and not with heart failure alone. 

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. The present study is the first one to analyse the class of drugs is similar than that of other hypoglycemic agents.

58 Reported incidence and severity of suspected abacavir hypersensitivity reactions (HSR) through at least 6 weeks in a large, controlled clinical trial using abacavir/3TC fixed dose combination (ABC/3TC FDC): The KLEAN study

Z. Anton*, M. Malbezin*, on behalf of the KLEAN Study Team *Marly le Roi – France*

Introduction: KLEAN was designed to compare the efficacy and safety of fosapenavir 700 mg BID + ritonavir 100 mg BID to Lopinavir/ritonavir BID both administered in combination with ABC/3TC FDC AND 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 usually mild hypersensitivity reactions have subsided.

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 2BID. Subjects with 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 occurred 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 (Grade 1). Twenty-four (2.7%) were moderate (Grade 2). Eighteen (2.0%) were severe (Grade 3 or 4) and two (0.2%) were very high dose. Two (0.2%) 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 maximum 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. Twenty-nine (2.7%) were moderate (Grade 2), 18 (2.0%) were severe (Grade 3 or 4). Any additional reports of ABC HSR will be reported with the final analyses.

59 Adverse reactions to meglumine antimoniate


Meglumine antimoniate (MA) is the first-choice drug of treatment for visceral leishmaniasis (VL) and for cutaneous leishmaniasis. The aim of our study was to analyse the type and the delay of onset of the adverse events due to MA, seen in 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 the adverse events described: 

- One case where the data were incomplete
- Three cases where the responsibility of MA was excluded because of incomplete delays.

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 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 choic 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 no cases of cardiac and renal failure with doses higher than the maximal daily dose recommended by the world health organisation (WHO) (max dose ≤ 850 mg/day). In these cases the delay of the event exceeded 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.

57 The effect of fluconazole on cutaneous toxicity related to intravenous sulframethoxazole trimethoprim in HIV patients with Pneumocystis carinii pneumonia

G. Jolimoy*, J. Dugue*, S. Logerot*, P. Chavanel, C. Sgro*, "Dijon – France"

Introduction: Cutaneous toxicity is frequent in human immunodeficiency virus-infected patients treated with sulframethoxazole trimethoprim (SMTP) for Pneumocystis carinii pneumonia (PCP). Risk factors of this hypersensitivity reaction are reported, including high posology, glutation depletion, slow acetylor phenotype of N acetyl transferase 2 and reactive metabolites overproduction toxicity. 

Conclusion: In 2004, Winter et al* 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 SMTP for PCP were included in this retrospective study. The cases were recruited in four hospitals. Characteristics of the patients, clinical presentation and adverse events were collected for each patient. The incidence of SMTP cutaneous hypersensitivity reaction was compared between patients treated with and without CYP2C9 inhibitor. 

Conclusion: Despite the small studied population, our results suggest that fluconazole should protect HIV-infected patients from cutaneous toxicity of SMH. 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 urinaiary metabolites dosage and analysis of acetylator phenotype.

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


Introduction: Multiple sclerosis (MS) is a demyelinating disease, interest 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 lymphocyte functions and central inflammatory process. 

Conclusion: 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 analysed 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 pharmacists’ prescription registry. We selected only patients who received a total dose superior to 70 mg/m². 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 treated with mitoxantrone. 24 patients, 15 men and 9 women, (29–60 years-old) had been treated with more than 70 mg/m². The mean follow-up was 4.3 years (from 18 months to 6 years). Past history of serious infections disease or familial MS was noted in 8 patients. 

Conclusion: 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 cardiac examinations have been recorded.
Acute overdosage with milnacipran
T Hacene, 1 F Boulanger, 1 J-F Bachelet, 1 J-P Jaulin, 1 M Guibert 1 and G Fazi 2

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: In an epidemiological case series 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 manufacturer Pierre Fabre Medicament.

Results: In 86% of the cases milnacipran was ingested with other drugs, in particular with benzodiazepines and alcohol. The adverse effects of acute overdose of milnacipran alone were not severe. Up to an intake of 1000 mg milnacipran was usually observed mainly of nausea and vomiting. Also at higher doses – the highest reported acute overdose of milnacipran only was 2800 mg – the symptoms observed were not more severe. It included mainly single vomiting episodes, loss of blood pressure, slight depression of respiration and sweating. Cardiotoxicity such as conduction abnormalities was not an issue.

Conclusion: Severe adverse effects are 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 mainly 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 between other drugs could be identified. On the contrary, the pharmacodynamic and pharmaco-kinetic properties of milnacipran speak against such a risk.

Serotonin-induced activation of TRPV-like current in rat intrapulmonary arteries: role in cell proliferation
T Hacene, F Boulanger, J-F Bachelet, J-P Jaulin, M Guibert and G Fazi

Introduction: 5-Hydroxytryptamine (5-HT) is involved in numerous biological processes in vascular tissue. In pulmonary arteries, 5-HT acts as a vasoconstrictor and mitogen in the regulation of sodium reabsorption in the kidney, thus playing a key role in the control of renal and blood pressure. Cardiovascular disease (CVD) is clearly established. Since the signal transduction pathways 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 between other drugs could be identified. On the contrary, the pharmacodynamic and pharmaco-kinetic properties of milnacipran speak against such a risk.

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Methods: After cell surface biotinylation, Western blotting was performed either on biotinylated or total proteins, leading to detection of total TrkA protein, respectively. The mechanism of TrkA internalisation was studied in HASMC transfected with wild-type (WT) or mutant (MUT) pincher plasmids (12 ng 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 degrada-
tion was studied in presence of the lysosomes inhibitors NH4Cl (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 for 4 days of NGF treatment.

Results: NGF induced TrkA internalisation in HASMC ( ~8 ± 2 % in surface TrkA at 15 min, P < 0.001). This internalisation increased in cells transfected with wild-type, WT ({P < 0.001}), and was progressively reduced in cells transfected cells (41 ± 4 %, P < 0.001). TrkA internalisation was partially blocked in clathrin sRNA transfected HASMC (47 ± 7 % inhibition, P < 0.001), control cells transfected cells were partially inhibited by MDC (58 ± 1 and 51 ± 3 % inhibition, respectively, P < 0.001). Immunofluores-
cence revealed clathrin recruitment to the cell membrane and co-immunopre-
cipitation 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 or 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 < 0.001), 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, which may have physiological and pathophysiological consequences in the airways.

Methods: The goal of the present study was to examine whether pharmacological decrease of PKR activity could be a possible target for therapeutic strategies in Alzheimer’s Disease. The PKR activity was measured in different cellular and animal models and in human lymphocytes of Alzheimer’s Disease patients with an activation of PKR and an inhibition of PKR phosphorylation with two PKR inhibitors: a chemical activator: a, and a selective agonist of group I metabotropic glutamate receptor, DHPG activators: a.

Introduction: Alzheimer’s disease is a neurodegenerative disorder of the central nervous system characterized by two major lesions: senile plaques composed of accumulated amyloid β peptide and neurofibrillary tangles (NFTs) 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) signaling pathways. mRNA transcription 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 PKR phosphorylation in mTOR/p70S6K 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/ p70S6K in these different cellular and animal models may affect neuroprotection in a cellular model of extracellular Aβ neurotoxicity.

Methods: Human (SH-SY5Y) neuroblastoma cells differentiated into mature neuronal cells were treated with different inhibitors of PKR phosphorylation or mTOR/p70S6K 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 p70S6K (Thr389) were analysed 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, PBL, is able to completely reverse cell 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 cell.

Concerning the mTOR/p70S6K pathway, the treatment with three potent activators: a-pioid receptor agonist, DAMGO, a branched-chain amino acid, L-leucine or a selective activator of group I metabotropic glutamate receptor, NR1, causes an increased phosphorylation of mTOR and/or p70S6K 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 an 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 Aβ toxicity.

Methods: Twenty-four hyperglycemic patients were submitted to an individualized exercise training program performed at an intensity corresponding to the Vo2peak and HADH activity did not change. A strong relationship was found between CS activity and Fatmax point (r= 0.58, P< 0.05).

Conclusion: We conclude that whole body substrate oxidation capacities and muscle oxidative capacities did not differ between D and over weight 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.

68 Swimming training reduces and stabilizes unstable atherosclerotic plaques in apolipoprotein E knockout mice (apoE-/-) with renovascular hyperten-
sion. M Pellegrin, J Aubert, K Boureauoune, P Laurant, A Berthelot, D Hayot, L Mazolli, Besançon – France – Lusseux – France

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 II (Ang II) levels in the brain of C57BL/6 mice (Ang II transgenic model). Normotensive 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-
aventral 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 (n=SMC immunostaining) and plaque inflammation (Mac-2 immunostain-
ing).

Results: 2K1C ApoE-/- mice developed significant hypertension compared to sham operated mice. Swimming did not reduce blood pressure. Swimming exercise significantly 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 plaques. 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 atherosclerotic plaques in hyperglycemic ApoE-/- mice. This finding suggest a new benefit of exercise training in the prevention of atherosclerosis.

69 Musclin gene expression is strongly related to fast-glycolytic phenotype in skeletal muscle. A Peinnequin, S Koulmann, H Sanchez, B Serrurier, E Habouzit, A Fenailleau, X Pigard, "La Trenche – France"

Introduction: Musclin has recently been described as a muscle-derived secreted molecule, 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 gene expression could depend on fibre type. Moreover, whether changes in muscle phenotype induced by exercise might be related to increased musclin gene expression in isolated plantaris fibers previously classified according to their MHC isoform content (I, Ia, Ix or Iib). Transition of muscle phenotype was obtained by hindlimb suspension (slow-to-fast transition in soleus muscle) or overexpression of MyoD (fast-to-slow transition in plantaris muscle), musclin gene transcription was measured.

Methods: The expression of Musclin mRNA was measured in soleus, plantaris and white gastrocnemius muscles of C2C12 and 3T3-L1 cells, using semiquantitative RT-PCR. The expression of Musclin mRNA was measured in isolated plantaris fibers previously classified according to their MHC isoform content (I, Ia, Ix or Iib). Transition of muscle phenotype was obtained by hindlimb suspension (slow-to-fast transition in soleus muscle) or overexpression of MyoD (fast-to-slow transition in plantaris muscle). Musclin gene transcription was measured.

Conclusion: The expression of Musclin mRNA was strongly, positively correlated with the MHC isoform content of fibers (r = 0.83, P< 0.001). The expression of Musclin mRNA was also strongly, positively correlated with the proportion of Ia fibers (r = 0.75, P< 0.001).

Methods: Twelve 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 (first phase area under the curve, AUC0-120 min) and basal insulin secretion (B). Plasma glucose and insulin levels were determined after 2 hours of an individualized training program performed at an intensity corresponding to the maximal rate of fat oxidation (Fatmax 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 increased only in C (P< 0.01). However, muscle and hydro-acyl dehydrogenase (HADH) were not significantly different between D and C. Training induces in D a significant increase of Fatmax point expressed in l/min (P< 0.05) while the rate of fatty oxidation at Fatmax point (fa(ox)) expressed in mg/min (P< 0.05) which provide evidence of a preferential use of fat during exercise. CS activity and mitochondrial capacity to oxidize palmitoyl-CoA + carnitine/medium and pyruvate + malate were also significantly increased after training in D (P< 0.05). SI, VO2 peak and HADH activity did not change. A strong relationship was found between CS activity and Fatmax point (r= 0.58, P< 0.05).

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.
and compared to control animals. Finally, we studied plantaris muscle transcriptomes in response to acute treadmill exercise or endurance training (1, 2 and 10 weeks).

Results: Muscle mRNA was detected at high levels in gastrocnemius and plantaris in response to acute exercise training. The single fiber analysis showed that muscle was produced by myofibers themselves, almost exclusively in type I fiber. Slow-to-fast transition in soleus phenotype induced by hindlimb unloading was associated with increased mRNA in both soleus and plantaris. The single fiber analysis showed that muscle was produced by myofibers themselves, almost exclusively in type I fiber.

Conclusion: Muscle 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 muscle expression. Major muscle phenotype transitions such as induced by unweighting or muscle overload are associated with changes in muscle expression. These results integrate muscle 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 muscle could play a role in the resistance of fast-glycolytic type Ib myofibers to the insulin-stimulated glucose uptake. Further studies are warranted to determine the exact mechanisms by which muscle could contribute to insulin resistance in fast-twitch glycolytic fibers.

70 Cardiovascular risk of recombinant human erythropoietin in trained rats with endothelial NO synthase inhibition

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Introduction: Cardioprotective effects of recombinant human erythropoietin (rHuEPO) can generate serious side effects. rHuEPO, by modulating directly endothelial function, or indirectly by increasing erythrocyte, blood viscosity and shear stress on the endothelial surface, is essential in the development of arterial hypertension (HTA) and arterial thrombosis. The presence of nitric oxide (NO) can protect from toxic (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, subcutaneously) or not with 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 each week. At the end of the protocol, an effort test was made. After sacrifice of rats, the citrate synthase activity was measured at the skeletal 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 training and 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<sub>max</sub> ≤ 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.

71 Effect of physical and mental stress on HR and HRV before and after exercise training among patients with cardiac diseases

S Carles, D Duran, A Pathak, G Roncalli, M Bousquet, J Garcia, JN Semard, M Galletier, I Malherbe & P Saint Orens – France

Introduction: Modifications of autonomic nervous activity, 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 realized during a mental stress using cognitive exercises, an acute exercise at 40% of maximal power, and their association.

Results: The acute stress 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 sympathic 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.

72 Renal hyporesponsiveness to brain natriuretic peptide: both generation and the 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 a pulmonary artery hypertension and eight matched control subjects participated in the study. After the baseline resting period (60; 10–10: 10 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.

Results: 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.

Conclusion: PH demonstrated an impaired ability to excrete the sodium load (2.2 ± % versus 40 ± 5%, P < 0.05) over the whole period. Plasma BNP was significantly increased in patients (64.7 ± 13.3 ng L<sup>−1</sup>) versus (10.9 ± 3.1 ng L<sup>−1</sup>, P < 0.01) in controls.

73 Abstract withdrawn

74 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) is a ligand to the epidermal growth factor receptor (EGFR). Previous studies have shown that 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 the 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 experimental crescentic glomerulonephritis.

Methods: Accelerated anti-glomerular basement membrane antibody-induced renal injury was performed in SV129 mice used as controls (CT), HB-EGF deficient mice (KO) and littermates treated with erlotinib or AG1478, pharmalogical inhibitors of EGFR tyrosine kinase activity (INH). Erlotinib was administered 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 (14.1 ± 1.0 and 10.5 ± 0.1 vs. 34.1 ± 7.2 mmol/L respectively for KO, INH and CT levels), lower albuminuria (8.5 ± 2.2 and 6.5 ± 2.0 vs. 9.4 ± 3.1 g/mmol creatinurina), lower percentage of crescentic glomeruli (32.6 ± 7.5 and 25.0 ± 6.9 vs. 62.8 ± 7.9 %) (P < 0.01 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 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 (immuno-chemistry).

Conclusion: These data provide evidence for the concept that immune-mediated glomerular injury leads to active and sustained pathophysiological recruitment of HB-EGF. The role of HB-EGF in crescentic glomerulonephritis may have potential implications in the regulation of renal inflammation, glomerular destruction and renal failure. The therapeutic potential of specific EGFR inhibitors may be envisioned in RPGN and other inflammatory glomerulonephritises.
75 Impact of ABC2C polymorphisms on methotrexate pharmacokinetics in patients with lymphoid malignancy

L Quteineh, N Simon, E Villard, D Fiallou, K Hoang-Xuan, V Leblond, P Lechat

Introduction: Human multidrug resistance protein 2 (MRP2, encoded by the ABC2C gene) is involved in the active efflux of anionic drugs such as methotrexate (MTX). A rare mutation in the ABC2C gene, the non-functional c.24C > T polymorphism, has been described in a patient with severe MTX elimination and subsequent toxicity. This study was conducted to assess whether more common ABC2C genetic variants can contribute to the variability of MTX pharmacokinetics and the onset of MTX adverse events.

Methods: A prospective pharmacogenetics study was conducted in 50 adult patients (mean age: 53 ± 17 years) receiving high-dose MTX (5.13 ± 1.88 mg/m² in 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 (non-linear 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 ABC2C 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). A multivariate linear regression analysis (stepwise selection including age, gender, MTX dose, renal function, and ABC2C genotypes) identified the −24T allele as a predictor of MTX concentrations at H48 (P < 0.005). Preliminary population PK analysis confirmed the significant influence of the −24T allele on MTX PK parameters. To analyse this influence, the −24T genotype carriers were compared with other patients who received MTX as a mono-chemotherapy 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 with other patients versus 40% respectively). None of the other studied polymorphism was associated with MTX pharmacokinetics in our study.

Conclusion: The −24T ABC2C polymorphism is associated with MTX pharmacokinetics. 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.

76 Influence of induction genetic polymorphism of CYP1A2 on pharmacoki- netic and pharmacodynamic parameters of clozapine: a pilot study

T Besnard, R Garralot, T Lavrut, D Allorge, MD Dréac

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 metabolism by p450, cytochrome P450 2D6 and 3A4. A polymorphism. To study the influence of CYP1A2, on pharmacokinetic and pharmacodynamic parameters of clozapine: a pilot study

Subjects (median age: 22 years) received oral clozapine (12.5 mg) at the beginning of treatment. Blood samples were collected 8 hours after administration for genetic and pharmacokinetic analysis. For both protocols, four microdialysis fibers were placed in forearm skin. During local heating (protocol 2), norclozapine plasma concentrations ratio (R2) was measured too. In parallel a visual attention test was used to estimate the cyclooxygenase (COX) pathway contributes to active vasodilation during local thermal hyperemia. For both protocols, four microdialysis fibers were placed in forearm skin. During local heating (protocol 2), norclozapine plasma concentrations ratio (R2) was measured too. In parallel a visual attention test was used to estimate the cyclooxygenase (COX) pathway contributes to active vasodilation during local thermal hyperemia.

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Hypersensitivity was prevented when DEX was added in culture medium (EC were assessed using a wire myograph and compared to responses from fresh J Favre

These results show that, 7 days of culture develop a hyper-sensitivity capacity of smooth muscle cell was still preserved. DEX exerts a beneficial effect on

Methods: We used the baseline data of 646 monophasic women, screened for the Causality double-blind placebo-controlled trial comparing atorvastatin (80 mg/day), versus placebo, ± with hormone therapy, on the progression of CCA-IMT and arterial stiffness. Aortic stiffness was measured by carotid pulse velocity (PWV, central and aortic), and a waveform index (AI, wave reflection) were determined by applanation tonometry; carotid stiffness was calculated from relative stroke change in diameter (echotrack system) and carotid PF

Results: BB were used in 104 women for treating headache, tachycardia, arrhythmia, and hypertension. 97% BB were used devoid of vasodilating properties. Age (63 ± 6 yrs, P = 0.0001) and mean BP ± 12 vs. 88 ± 11 mmHg, P < 0.0001) were slightly but significantly higher in BB+ than in BB- group (P < 0.0001). Age in both groups had 10% higher P (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-BB+ group. Despite a non significantly higher brachial AI only, (P = 0.001), we observed an influence of hyper-tension and hyper-tensive, 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 monophasic women with hypertension 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 TP study. 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 relaxa-

tion in a mouse model of cardiomyocyte-specific overexpression of aldosterone 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; the absence and in the presence of the BKCa inhibitor ibetiront, and the relaxing responses to the BKCa activator NS-1619 were also assessed. BKCa-α and β1 subunits expression were quantified in microwave 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 BB+ vs. WT. Furthermore, BB+ subcutaneous histamine was 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 EDRF-mediated response may contribute to the deleterious effect of aldosterone in cardiovascular diseases.

84

Postocclusive reactive hyperemia inversely correlates with urinary 15-F2t-

Isoprostane levels in systemic sclerosis. A12-month double-blind randomized trial comparing the effects of the angiotensin-2 receptor antagonist losartan (50 mg/day) vs. placebo in a12-month double-blind randomized trial comparing the effects of the angiotensin-2 receptor antagonist losartan (50 mg/day) vs. placebo in

Methods: We enrolled 43 patients suffering from systemic sclerosis, 31 patients (mean age 43, Raynaud’s phenomenon (RP), and 25 healthy volunteers. Microvascular function was assessed using the postocclusive hyperemia measured by laser Doppler flowmetry. Endothelium-dependent response was monitored after 3, 6 and 9 months. Individual nitroglycerin + acetylcholine 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 postocclusive hyperemia and primary RP compared to healthy volunteers. Microvascular function was assessed using the postocclusive hyperemia measured by laser Doppler flowmetry. Endothelium-dependent response was monitored after 3, 6 and 9 months. Individual nitroglycerin + acetylcholine 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 postocclusive hyperemia and primary RP compared to healthy volunteers. Microvascular function was assessed using the postocclusive hyperemia measured by laser Doppler flowmetry. Endothelium-dependent response was monitored after 3, 6 and 9 months. Individual nitroglycerin + acetylcholine was assessed by urinary levels of the F2-isoprostane 15-F2t-isop using GC–MS.

Conclusion: 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 postocclusive hyperemia and urinary levels of the F2-isoprostane 15-F2t-isop in patients suffering from systemic sclerosis.

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Results: Mean pollutant levels were more closely correlated with endothelial parameters (P = 0.0005). Baseline dSS (P = 0.0005) was significantly and negatively correlated with NO (P = 0.0005), S02 (P = 2.10^-5) and JO SO2 (P = 2.10^-5), CO (P = 7.10^-4) and JO CO (P = 0.0008). SPO2 (P = 0.0001). Expl. var. 19% of the variance of baseline dSS and dVel were significantly correlated with PM 2.5 (P = 0.02) and PM10 (P = 0.001). (2) Changes in dBr between the two visit was significantly correlated with delta NO (P = 0.001) and delta SPO (P = 0.0001). (3) A significant and positive correlation between 8% of the variance of baseline dBr and dVel in diameter or shear stress after TNT were not correlated with changes in air pollution parameters.

Conclusion: Endothelial function is significantly impaired by ordinary level of pollutant in healthy young males, in an urban area. Gaseous pollutants influence large artery endothelium whereas particulate matters influence small artery responses.

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

Y Leblaisa, E Delannoya, B Hugueretb, A Gaduca, C Desengoc, R Marthanb, M Mullerb
a Bordeaux – France; b Villejuif – France

Introduction: We have previously shown that in mice pulmonary arteries, the β2-adenoreceptor 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 role of eNOS in the β2-adenoreceptor function in mice pulmonary arteries (i); to evaluate the role of different protein kinases (which are described to phosphorylate the endothelial NOS (eNOS) and regulate its activity) in the β2-adenoreceptor-mediated relaxation; and (ii) to investigate the β2-adenoreceptor-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 β2-adrenergic agonist (procaterol) was evaluated after submaximal precontraction with prostaglandin F2α. Immunohistochemistry experiments were also performed on mice pulmonary arteries sections using a polyclonal anti-β2-adrenergic antibody.

Results: Immunohistochemistry experiments show a β2-adrenergic receptor 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, 100 μM), but not modified in the presence of the protein kinase A (PKA) inhibitor (Rp-8-Br-cAMPS, 100 μM), the protein kinase C (PKC) inhibitor (staurosporine, 10 μM) and the protein kinase G (PKG) inhibitor (H-89, 1 μM).

Conclusion: These data demonstrate that the β2-adrenergic receptor is functionally expressed in endothelial cells of mice pulmonary arteries. They suggest that the β2-adrenergic receptor coupling to eNOS is independent of PKA, PKC or PKG pathways. Furthermore, they show that genetic deletion of eNOS promotes a switching of the β2-adrenergic receptor coupling from eNOS to an EDHF- and/or cGMP-stimulated relaxant pathway.

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

E Naline, A Buenestado, S Grasind Delyl, C Advenier, P Devillier "Suresnes – France

Introduction: The current study was performed to investigate whether cannabinoids (CB) receptor agonists (WIN55,212-2; CB1/CB2 agonist and WIN11131; CB2 agonist) could alter the basal tone of isolated human bronchi. These vessels were mounted in a wire myograph containing methylene blue. Henseleit solution containing indomethacin (10^-5 M) and a CysLT1 receptor antagonist, MK476 (10^-6 M). EFS (biphasic pulse width 1 ms, constant current of 320 μA) was used to induce contraction in the absence of ACh (10^-4 M) which was monitored for 240 min. ACh-induced contraction was studied by cumulative addition of increasing concentrations of ACh (10^-6 M to 3x10^-7 M). Human extrapulmonary arteries incubated with WIN55,212-2 or JWH-133 (3x10^-7 M) for 30 min before EFS- or ACh-induced contractions.

Results: The cannabinoid agonists had no direct effect on basal tone and did not modify the ACh-induced contraction whereas the two CB2 receptor antagonists 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 JH1-133 with dual agonist activity for the CB1/CB2 receptors. In addition, the inhibitory effect of WIN55,212-2 was reversed by g-1-hor incubation with the CB1 receptor-selective antagonist (SR141716: 10^-6 M) but not with the CB2 receptor-selective antagonist (SR144528: 10^-6 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.

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


Introduction: Common carotid artery intima media thickness (CCA-IMT), aortic pulse pressure (PP) and central pulse wave velocity (PWV) are early markers of atherosclerosis. The influence of hormonal replacement treatment (HRT) on arterial parameters in postmenopausal women remains to be investigated.

Methods: We evaluated baseline aortic and extralobar pulmonary arteries 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, central PWV, PP and PWV. Aortic stiffness was determined using a high-definition echotracking device (Esaot ®), aplanography technology (SphygmoCor ®), and CrossCorr ® 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.

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

Y Leblais, F Fresquet, Y Leblais, R Marthan, M Muller "Bordeaux 2 – France

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 hypoxic 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 immunohistochemistry experiments were also performed on mice pulmonary arteries sections using a polyclonal anti-β2-adrenergic antibody.

Results: Immunohistochemistry experiments show a β2-adrenergic receptor 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, 100 μM), but not modified in the presence of the protein kinase A (PKA) inhibitor (Rp-8-Br-cAMPS, 100 μM), the protein kinase C (PKC) inhibitor (staurosporine, 10 μM) and the protein kinase G (PKG) inhibitor (H-89, 1 μM).

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80 Hormone therapy and risk of venous thromboembolism among postmenopausal women. Influence of the Cytochrome P450 1A2 genetic polymorphism (CY1A2*1F)

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

Independent determinant CCA-IMT (μm) Central PW (mmHg) PWV (m/s)

<table>
<thead>
<tr>
<th>b</th>
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<th>b</th>
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<tbody>
<tr>
<td>Age at M (5 y•r)</td>
<td>25.9</td>
<td>&lt;0.001</td>
<td>3.1</td>
<td>0.0004</td>
<td>0.4</td>
</tr>
<tr>
<td>(5 years)</td>
<td>25.0</td>
<td>&lt;0.001</td>
<td>3.1</td>
<td>0.0004</td>
<td>0.4</td>
</tr>
<tr>
<td>Δendothelial height (μm)</td>
<td>-3.7</td>
<td>0.001</td>
<td>0.9</td>
<td>0.0001</td>
<td>0.4</td>
</tr>
<tr>
<td>Mean BP (10 mmHg)</td>
<td>7.0</td>
<td>0.002</td>
<td>0.4</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>Central PP (mmHg)</td>
<td>9.1</td>
<td>0.004</td>
<td>2.7</td>
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<tr>
<td>Total PWV (m/s)</td>
<td>13.2</td>
<td>0.001</td>
<td>4.8</td>
<td>0.0001</td>
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k increment of % explained variance; bbope: slope of the multivariate correlation.

Conclusion: Duration and age at menopause were associated with thickening and stiffening of large arteries. Changes in parameters of CCA-IMT were significantly different between HRT and HRT users.

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The cytochrome P450 1A2 (CYP1A2) is partly responsible for the metabolism of estrogenic and many exogenous compounds including caffeine. A single nucleotide polymorphism (SNP) in intron 1 (−16.16 C → A) of the CYP1A2 gene (CYP1A2*1F) influences the extent to which CYP1A2 is induced in cigarette smokers. CYP1A2*1F allele is more frequently observed in cigarette 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 450 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 = 1.8: 95% CI: 2.3–6.5 and OR = 0.8 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: 0.5–34.7) 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.

91 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 CH mice (11–13 week-old) were exposed to either normoxia (0.5 atm for 21 days) or treatment to CH mice (15 ± 2 vs. 2 ± 3% P = 0.05 in CH and control, respectively). The NO synthase inhibitor, N-nitro-L-arginine methylster (L-NAME), at a concentration (300 μM) which totally abolished the endothelium-dependent relaxant effect of acetylcholine, did not modify the myogenic response. The NO production was evaluated by measuring nitrite and nitrate (NOx).

Results: In normoxic mice, myogenic tone was present in mice of both groups. In hypoxic mice, the contribution of NO to the myogenic tone was reduced significantly whereas the NOx production was increased. Furthermore, this pressure-dependent contractile response is NO- and endothelium-dependent. NO is produced by the endothelium and is transported to the media via a paracrine mechanism to modulate smooth muscle contraction and relaxation.

Conclusion: These data 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, NO cannot be attributed to a rise in myogenic tone in this PA.

94 Proportion of the antihypertensive treatment effect on the relative reduction of cardio-vascular risk explained by blood pressure
S Gerber*, F Gueyffier*, JM Wrighst, A Belot*, P Roy* Lyon – France; bVancouver – Canada

Introduction: The object 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 cardio-vascular (CV) risk factor (i.e. differences explained 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 to last 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 has been 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.

95 Cross-link between PI3-kinase and MAP kinase pathways in the regulation of NO signaling and increase in reactive oxygen species production by acute hypoxia
I Descamps, B Muller*, R Marthan, F Bourgeade** Bordeaux 2 – France

Introduction: Chronic hypoxia (CH) induces increase in pulmonary vascular resistance (PVR), and leukocyte infiltration. Hypoxia results in potential arterial remodeling with the major 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 C57BL/6J mice were exposed or not to hypoxic 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 0.05 atm, the pressure present was 3.5 ± 1.3 and 1.7 ± 0.7 mmHg in control and CH mice, respectively, a 30% reduction in the contractile response was observed in PA from CH mice (15 ± 2 vs. 2 ± 3% P = 0.05 in CH and control, respectively). The NO synthase inhibitor, N-nitro-L-arginine methylster (L-NAME), at a concentration (300 μM) which totally abolished the endothelium-dependent relaxant effect of acetylcholine, did not modify the myogenic response.

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, NO cannot be attributed to a rise in myogenic tone in this PA.

92 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 arteries in a well defined model of apolipoprotein E knockout (apoE−/−) mice with CRF.

Methods: We measured systemic mean arterial pressure (MAP, mmHg) and cerebral arterial internal diameter (ID, μm) in anesthetized apoE−/− and C57BL/6 J mice (WT) with CRF induced by electrocauterisation of the right kidney followed by controlateral left nephrectomy). We examined autoregulation-induced vasodilatation (AV, μm) by measuring ID prior to and during stepwise hypotension (50 mmHg per stage), which was also measured during a second stepwise hypotension after complete deactivation of cerebral arteries with EDTA (67 mmol/L).

Results: Baseline values were expressed as mean ± SEM (P<0.05 vs. WT, *P<0.05 vs. sham, two ways ANOVA). ID was measured at a MAP of 30–40 mmHg.

Conclusion: Our results suggest that cerebral arteries might undergo vascular remodeling during CRF, which might impair AVH.

WT (n=11) CRF (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
ID, μm 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, AV was impaired in WT and apoE−/− mice with CRF.
Methods: MPs were produced by activation of human lymphoid CEM T cell line with the agonistic agent, anti-CD3/anti-CD28 antibodies. In Eahy 926 endothelial cells were grown for 24 h in absence or presence of 10 μg/mL microparticles pre-incubated or not either with PK-i kinase inhibitor (LY294002, 20 μM), MEK 1/2 inhibitor (U0126, 10 μM), or 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) followed by post hoc Tukey for multiple comparisons. 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 (91 ± 4%) and decreased its phosphorylation by Tyros 14 (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 PK-i, LY294002, reduced the effects of MPs on eNOS but not on caveolin pathways whereas it potentiated the effects of ROS production and another set of endothelial MPs stimulated the ERK1/2 phosphorylation (73 ± 7%). As expected the MEK inhibitor, U0126, prevented ERK1/2 phosphorylation (48 ± 5%). Interestingly, U0126 reversed eNOS phosphorylation (Ser1177 and Thr495 60 ± 5, 85 ± 5% 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 PK-i kinase. Beside, PK-i 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 upregulated oxidative stress in the vessel wall. The latter may account for the deleterious effects of MPs resulting in endothelial dysfunction.

96 5-Loxygenase 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-Loxygenase-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 the LTs production by polymorphonuclear leukocytes stimulated either with A23187 or arachidonic acid, 2/3 the urinary excretion of LTB4 and, 3/ the relationship between LTs severity and LT production.

Methods: We prospectively studied 56 OSAS patients and 16 control subjects. LTB4 and LTA4 were quantified by liquid chromatography tandem mass spectrometry.

Results: LTs production (expressed as ng of LT/μg of DNA, or ng of LT/μmol of arachidonic acid (30 μM) was significantly increased in OSAS patients compared to controls. Moreover, the production of LTA4 in response to A23187 or arachidonic acid was correlated with the arterial nocturnal oxygen desaturation (SaO2, p = 0.056, P = 0.001). LTB4 excretion was also higher (P = 0.005) in OSAS patients in multivariate analysis. LTA4 levels were associated with SaO2 independently of confounding factors such as age or body mass index (BMI) whereas LTB4 levels were correlated to BMI.

Conclusion: The activation of the two arms of the 5-Loxygenase 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.

97 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 pulmonary hypertension (PH). We previously studied 56 OSAS patients and 16 control subjects. LTi and LTc were quantified by liquid chromatography tandem mass spectrometry.

Methods: Male C57BL/6 mice (10–12 week-old) were exposed or not to hypobaric hypoxia (21% O2, 5% CO2, 94% N2) in a exposure chamber, for 10–12 weeks. After exposure, animals were euthanized, and pulmonary arteries were removed and mounted on a wire myograph, for evaluation of contractile responses to receptor-dependent (phosphoryline, and ß, adrenergic agonist) and receptor-independent (depolarizing KCl) agents.

Results: Nociceptins and endovanilloids induced by KCl and phenylephrine were markedly enhanced in pulmonary arteries from mice exposed to hypoxia, compared to controls (1.5–1.7 fold increase in maximal effect). In pulmonary arteries from hypoxic mice, contractile effect of phosphoryline was significantly diminished in presence of the selective cyclooxygenase-1 inhibitor (SC561, 1 μM), selective cyclooxygenase-2 inhibitor (NS398, 1 μM), and thromboxane A2 receptor (TP) antagonist (SQ29548, 0.5 μM; or L670562, 1 μM). However, in these hypoxic pulmonary arteries, the thromboxane A2 receptor antagonist (fagaradate, 10 μM or 100 μM) did not modify significantly phosphoryline-induced contraction. Catalase (250 U/mL), which decomposes hydrogen peroxide, decreased hypoxic pulmonary arterial vasoconstriction in mice exposed to hypoxia. Combination of catalase and L670562 did not induce greater inhibition of contraction than catalase or L670562 alone in these arteries. None of these agents affected the contractile effect of phosphoryline in pulmonary arteries from control mice.

Conclusion: These data provide evidence that, in mice pulmonary arteries, hypoxic exposure sensitized vasoconstrictor responsiveness to thromboxane A2. These results suggest the existence of 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 A2 is likely responsible for exacerbated vasoconstriction.

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98 Digital thermal hyperaemia impairment does not relate to skin fibrosis or macrovascular disease in systemic sclerosis

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 21 healthy volunteers.

Results: In the first study, the 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 second study, seven SSc patients of age 44 ± 11 years, seven of them with a limited cutaneous involvement in terms of skin fibrosis, in comparison with healthy volunteers, we observed a clear-cut decrease in digital systolic blood pressure at 44°C.

Conclusion: In patients with SSc, digital thermal hyperemia is impaired, but doesn’t relate to the skin fibrosis or to an associated macroangiopathy. Further studies are required to determine whether its impairment reflects a functional or structural microvascular damage.
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 renal vascular resistance changes. The substances were injected as a bolus of 5 µL (dose expressed in mL) or infused (concentration expressed in µM).

Results: Increasing doses of ET-1 and sarafotoxin 6c (ET receptor agonist) caused potent vasoconstrictions with a maximal amplitude of 183 ± 7 and 116 ± 13 mmHg and an EC50 of 6.2 and 2.6 pM, respectively. At the dose of 5 pM, the constrictions to ET-1 showed a long duration of action (>30 min) while those to sarafotoxin 6c (10 pM) were transient. ET-1-induced constrictions were partially inhibited by BQ-123 (0.1 and 1 µM) or by R-788 (0.1 and 1 µM) but completely blocked by the combination of the two antagonists. Statistical analysis showed significant (p<0.05) and dose-dependent responses in the absence 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 significantly reduced by the combination of the two antagonists. Statistical analysis showed significant (p<0.05) and dose-dependent responses in the absence 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 significantly reduced by the combination of the two antagonists. These findings show that in the isolated perfused mouse kidney, ET-1 is a potent vasoconstrictor agent that induces constrictions by stimulating both ETA and ETB receptors present at the level of the vascular smooth muscle. The data also suggest that stimulation of ETB receptors is responsible for the long duration of the ET-1 response, while the activation of the both receptors is responsible for the initial fast constrictions. These findings were supported by the endothelium-mediated responses to ET-1 and sarafotoxin 6c, which were not involved in this model. This study illustrates the important contribution of both ETB and ETA receptors in the renal resistance vessels in the mouse.

101 Pulse wave velocity as independent predictor of subclinical atherosclerosis burden

Methods: Compared to traditional risk factors, Pulse wave velocity (PWV), the sclerotic component of athero-sclerosis, is closely dependent of blood pressure (BP) and age, but it has been suggested that its elevation provide incremental prognostic information as an independent risk factor. Pulse wave velocity was measured mecanographically with an automatic detection. Plaque burden was deduced from both sides of common carotid artery. Carotid-femoral PWV was measured from femoral sites and intima-media thickness (IMT) measurement. Mean IMT was calculated using ultrasound detection of plaque at carotid, aorta and femoral sites.

The study was performed with a hospital based cohort of 646 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 measurement of arterial wall-thickness. PWV and IMT were measured as an index of aortic stiffness and subclinical atherosclerosis severity. The values of PWV and IMT were compared between groups. Statistical analysis were performed by a one way analysis of variance (ANOVA), and Mann–Whitney U tests or tow way ANOVA for repeated measurements 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 a bradycardin-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 Pl3-kinase and ERK signalling were specifically inhibited, the effects of microparticles were reversed. In vivo injection of microparticles in mice induced a significant vascular hyporeactivity suggested to participate in CD patients.

Conclusion: Taken together, we propose that the biological message carried by microparticles may help in understanding the vascular changes that occur in CD patients and may give a potential therapeutic target for CD patients.

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

Methods: The present 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. The mechanism of vascular smooth muscle cell was recorded in isolated carotid artery strips with intracellular microelectrode.

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

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

Methods: Microparticles were isolated following serial centrifugations. Early 92 endothelial cells were grown for 24 h in absence of and in presence of 10 µg/mL microparticles pre-incubated or not with PI3-kinase inhibitor (LY294002, 20 µM). MEK 1/2 inhibitor (U0126, 10 µM), cyclosporine (30 µM), a specific antagonist of the Hedgehog receptor (Patched), or siRNA of Patched. Cells 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 ring. Statistical analysis showed significant (p<0.05) dose-dependent responses in the absence 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 significantly reduced by the combination of the two antagonists. These findings show that in the isolated perfused mouse kidney, ET-1 is a potent vasoconstrictor agent that induces constrictions by stimulating both ETA and ETB receptors present at the level of the vascular smooth muscle. The data also suggest that stimulation of ETB receptors is responsible for the long duration of the ET-1 response, while the activation of the both receptors is responsible for the initial fast constrictions. These findings were supported by the endothelium-mediated responses to ET-1 and sarafotoxin 6c, which were not involved in this model. This study illustrates the important contribution of both ETB and ETA receptors in the renal resistance vessels in the mouse.

104 T lymphocyte-derived microparticles from Crohn’s disease patients with active inflammatory disease induce vascular hyperactivity through a PPAR-gamma-dependent pathway

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. Mean IMT was calculated using ultrasound detection of plaque at carotid, aorta and femoral sites and measurement of arterial wall-thickness. PWV and IMT were measured as an index of aortic stiffness and subclinical atherosclerosis severity. The values of PWV and IMT were compared between groups. Statistical analysis were performed by a one way analysis of variance (ANOVA), and Mann–Whitney U tests or tow way ANOVA for repeated measurements 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 a bradycardin-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 Pl3-kinase and ERK signalling were specifically inhibited, the effects of microparticles were reversed. In vivo injection of microparticles in mice induced a significant vascular hyporeactivity suggested to participate in CD patients.
**Methods and Results:** C57BL/6J mice were exposed to intermittent hypoxia or normoxia for 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 hypoxia 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, measurement of 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 postocclusive reactive hyperemia in healthy volunteers**

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**Introduction:** Post occlusive and local thermal hyperemia are currently used as microvascular markers. The major limitation remains the microinvasive approach as local anaesthesia prior to fibre insertion could lead to confounding effects. The objective of our study was to determine whether EMLA® cream treatment (lidocaïne prilocaine) applied on the ventral side of the left upper forearm for 2 or 40 minutes 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 was treated at the start of functional testing and cream was placed on another site, followed 20 min later by cream placement on a third site. One hour before the start of functional testing, 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. One hour before the start of functional testing, 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. 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 and were 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).

**Conclusion:** EMLA® cream, when applied during 40 min, induces a significant 75% decrease of the following intradermal needle insertions, while not modifying skin postocclusive and thermal hyperemia 2 h after cream removal. Therefore, we recommend its use in such conditions before performing microdialysis mounted in a wire myograph (responses to acetylcholine, Ach), while small mesenteric resistance arteries were culminated to evaluate flow-mediated dilatation (FMD).

**Results:** Figure shows that IVA improved endothelial dysfunction in coronary and mesenteric (mesenteric) arteries isolated from CHF rats (**P=0.01** vs. CHF). These effects were abolished by the NOS inhibitor L-NAME (**10-4 M**). In normoxia treated CHF coronary arteries (in the presence of L-NAME 10-5 M), Ach induced endothelin-dependent, endothelium-dependent, contractions that were not affected by IVA. In CHF mesenteric arteries, the cyclooxygenase inhibitor diclofenac **10-4 M** improved FMD to a similar extent in untreated and IVA-treated rats.

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**Cutaneous vascular responses to hypoxia, hypercapnia, and hyperpnea in healthy volunteers**

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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 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 CO2), 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 vasodilatation 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.

**110**

**Effects of hyperlipidemic diet (egg yolk) and hypothyroid drug (carbamazepine) on atherosclerosis**


**Introduction:** Pseudomonas 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 nutritional modulations (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 a normal laboratory diet and the 2nd group was maintained on a high fat diet (50% of fat in the diet). The 3rd group received daily activity diet (50 g/day) and 3 g egg yolk (6 months). The 3rd group received daily standard laboratory diet (10 g/day) added with carbimazole (0.03%) dissolved in 0.09% NaCl. Moreover (5 months), thyroid gland, aorta, liver and kidneys were evaluated. The plasmatic parameters is effectuated by the enzymatic method and electrophoretic analysis of lipoproteins by horizontal gel electrophoresis. For histological examination, the aorta and liver was stained with hematoxylin and eosin, DNA and RNA were detected with acridine orange-hoechst and DNA and RNA were detected with acridine orange-hoechst and DNA.

**Results:** Hyperlipidemia and hypercholesterolemia are registered in the two groups of experimental Psammomys but are more pronounced in Psammomys on natural diet with egg yolk, cholesterol showed in this group a significant increase in the rat aorta. Aortic intima-media thickness (IMT) showed a significant alteration in the rat aorta.

**Conclusion:** Results, showed an increase of IMT in experimental Psammomys blood aggregation, phenotypic modulation of aSMC, collagen expression, vascular hyporeactivity but not endothelial dysfunction in the aorta via in vitro.

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**Napoli – Italy**

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

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**Introduction:** Hyperlipidemic diet induced by Carbimazole is confirmed by histological of thyroid study, this show an increase of mass of thyroid gland. Histologically, the enlargement is characterized by proliferation of follicular epithelium cells and the components of extracellular matrixes.

At the end of experimental period, plasma cholesterol and triglyceride levels as well as significant alterations in the rat aorta.

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**Napoli – Italy**

112 Interaction of myoin light chain kinase 210 with NF-Xb pathway in endothelial cells: evidence of a new cardiometabolic risk factor. FMD was the percentage of the maximum hyperemic vasodilation measurement providing a continuous evolution of the diameter during acute hyperemia was limited by operator dependence of most measurement methods.

**Introduction:** Flow-mediated vasodilation (FMD) of the brachial artery (BA), a non invasive marker of endothelial function in humans, is widely accepted for evaluating cardiovascular risk in healthy people and in patients with cardiovascular disease. Clinical use is limited by operator dependence of most measurement methods.

**Methods:** A new home-made automated computerized analysis of BA ultrasonograms permits 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 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), 1 week or 1 month apart (long-term).

**Results:** Thanks to very low coefficients of variation of diameter measurements (<2% for within reading, 4–8% for within subjects variability). FMD variability was as low as 7% on average for within reading and 8–18% for within subject variability. Short- and long-term FMD variabilities were twice higher in at risk subjects than in healthy volunteers.

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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 routine in patients with diseases associated with endothelial dysfunction.

A simple calculation indicates that, for a parallel group or a cross-over design, our new method requires two to three less subjects to include than conventional methods.

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115 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) and carotid intima-media pulse pressure (PP) in HIV+ patients with hypercholesterolemia (LDL-cholesterol >160 mg/dl) treated with HAART (>12 months). With a predefined calculation of the sample size, 42 patients treated with P (>12 months) and 42 age, sex, and smoking status-matched hypercholesteremic patients without lipid-lowering treatment were determined using invasive-high-definition echotracking device and applanation tonometry in a central core laboratory blinded to treatment.

Results: The groups were similar for both baseline characteristics including cardiovascular risk factors and IMT parameters. Mean duration of HIV infection was similar among P and P' and P' treated patients 12 ± 4 vs. 11 ± 5 years, P = 0.24. The mean duration of dyslipidemia was higher in the P group 5.0 ± 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. 9.8 ± 1.8 m/s, P = 0.25).

Using logistic regression, determinants of IMT were age and carotid PP whereas aortic stiffness was determined using pulse wave velocity (PWV).

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 determine aortic stiffness.

116 Circulating microparticles from septic shock patients modulate tissue 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 tissueular protein expression.

Methods: MPs were extracted from whole blood of septic (n = 52) and normal (n = 16) subjects that were utilised 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 (O2-) measurements by electronic paramagnetic resonance. (O2-) Measurements were determined by electronic paramagnetic resonance.

Results: O2 production was greater in heart and lung (2.3 and 2.8 fold respectively) than in liver, kidneys, and spleen of infected mice. MPs that were isolated from septic mice and healthy subjects were analyzed for NO and superoxide anion (O2-) measurements by electronic paramagnetic resonance. (O2-) Measurements were determined by electronic paramagnetic resonance.

Conclusion: Our results demonstrated significant differences in the two groups except that of liver in which septic MPs treatment induced 2-fold increase of NO production between mouse aortas treated with MPs from septic or healthy subjects [controls]. Quantitative measurement of endothelial cells (ECs) in blood was performed by the method according to Hladovec (1) based on counting of ECs in microsized particles, 42 patients treated with P (n = 42) and septic MPs, (n = 16) respectively, were increased in septic patients. NO difference in NO or O2 production between mouse aortas treated with MPs from either septic or healthy subjects was increased in aorta taken from mouse treated with septic MPs (EC50: 75 ± 2.1 x 10-6 M) (n = 7) compared to those with healthy subjects (EC50: 480 ± 090 M) t(n = 6) (P = 0.01). This effect was not modified in presence either of NO-synthase inhibitor, nitro-l-arginine (L-Arg) or 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 = 12) or healthy 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 (O2-) were measured by electronic paramagnetic resonance technique (ERP). MPs either from septic or healthy subject were injected i.v to mice and then, vascular reactivity was assessed in aorta by myograph. 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 ± 3700) (P < 0.01). Endothelial-d (192.3 ± 35.6 % reduction) (P < 0.05) and platelet-derived MPs (13290 ± 2643 vs. 8840 ± 3753) (P < 0.05), as well as P-selectin (4 (1.04 ± 12.6 vs. 48.6 ± 2.4)) and L-selectin’s MPs (6.14 ± 16.4 vs. 5.7 ± 5.5) (P < 0.05, P < 0.01, respectively) were increased in septic patients. No difference in NO or O2- production between mouse aortas treated with MPs from either septic or healthy subjects. Interestingly, the sensitivity of contraction in aorta to serotonin was increased in aorta taken from mice treated with septic MPs (EC50: 75 ± 2.1 x 10-6 M) (n = 7) compared to those with healthy subjects (EC50: 480 ± 090 M) t(n = 6) (P = 0.01). This effect was not modified in presence either of NO-synthase inhibitor, nitro-l-arginine (L-Arg) or 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 CaCl2 on arteries exposed to KCl-depolarization (n = 3) and relaxation to the Rho-kinase inhibitor n (n = 4) on vessels precontracted with phenylephrine U46619 were identical in aorta taken from healthy or septic-treated mice.

In conclusion, we demonstrated that these 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 vasoco- stritor metabolites from COX-1. The mechanisms implicated were not linked to enhanced calcium entry or sensitization through the Rho-kinase pathway. Thus, septic MPs may be rather protector against vascular hyperreactivity 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 investigations of 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, BIR1 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 TAT-BIR3/RING, could be used to enhance the protective agent against reperfusion injury.

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 carries Tat, a potent transmembrane protein of HIV-1, required for viral transduction. After anesthesia, 6–8 weeks-old C57/B16 mice were subjected to 30 min coronary artery occlusion followed by 24 h reperfusion (CAR). Intravenous injections of saline (control) or 0.8 μg/g of PTD-BIR3/RING were performed at 30 min, 1 h or 6 h after the onset of CAR. Caspase activities and the densities of several pro- and apoptotic proteins were assessed.

Results: BIR3/RING added 44 ± 2% 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 during reperfusion. These results also demonstrate 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 functional protective effect of isolated working mice heart model after in vitro 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 cancers. However, their clinical use of these drugs is limited by a high incidence of cardiotoxicity. Using the cardiomyocyte model described in the literature (single dose, i.p., 20 mg/kg), we aimed to assess the effect of anthracyclines administration on the cardiac function evaluated by an isolated working mouse heart method.

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 to 15 min to 0.05 μM PLP bracketed for 25 min with antagonists of P2 receptors (suramin, PPADS, APMP, MRS2578), 1 μM of the PKA inhibitor H89 and PLC blocker (U73122), 1 μM of the CaMKII inhibitor KN93 or a combination of the three (H89+U73122+KN93), 5 or 10 μM of PLP, or a combination of the PKA inhibitor with either glibenclamide (1 μM), 5-hydroxydecanoic acid (5-HD, 100 μM), U71122 (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, suramin, PPADS, APMP, MRS2578, 5-HD or a combination of the three. Conversely, H89 or KN93 or a combination with either glibenclamide (1 μM), 5-hydroxydecanoic acid (5-HD, 100 μM), U71122 (0.5 μM), H89 (1 μM) or KN93 (1 μM) were without significant effects on RPP. P2 antagonists, 5-HD, a mitochondrial selective K+ channel blocker suppress the protective effect on myocardial injury. The suppression of the cardioprotective effects of PLP by AMPs, the PKA inhibitor H89 and the phospholipase C blocker U73122 together evidence that PLP-induced cardiac preconditioning is a CaMKII-dependent process. The PKA inhibitor with either glibenclamide (1 μM), 5-hydroxydecanoic acid (5-HD, 100 μM), U71122 (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.

Conclusion: Recovery of RPP, measured 15 min after reperfusion, was improved by PLP, suramin, PPADS, APMP, MRS2578, 5-HD or a combination of the three. Conversely, H89 or KN93 or a combination with either glibenclamide (1 μM), 5-hydroxydecanoic acid (5-HD, 100 μM), U71122 (0.5 μM), H89 (1 μM) or KN93 (1 μM) were without significant effects on RPP. P2 antagonists, 5-HD, a mitochondrial selective K+ channel blocker suppress the protective effect on myocardial injury. The suppression of the cardioprotective effects of PLP by AMPs, the PKA inhibitor H89 and the phospholipase C blocker U73122 together evidence that PLP-induced cardiac preconditioning is a CaMKII-dependent process. The PKA inhibitor with either glibenclamide (1 μM), 5-hydroxydecanoic acid (5-HD, 100 μM), U71122 (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.

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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 control NZW rabbits. At the end of the treatment, total selenium was measured in the blood and the heart, liver and kidneys of all animals. In addition, the hearts were examined histologically.

Results: Total selenium was significantly (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 selenium levels did not differ significantly between experimental and control groups. In treated animals, developed heart lesions including disorganization of cardiac fibers, myositis, interstitial and endocardial fibrosis, and necrosis.

Conclusion: Our results support the hypothesis that 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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Objective: The present meta-analysis was performed to determine the cardiac effects of somatostatin analogs, i.e., octreotide (OCT) and lanreotide (LNR), using 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 the difference between acromegaly patients and normal controls and did not attempt to assess effect size.

Results: Somatostatin analog treatment was associated with a significant decrease in blood pressure and heart rate (HR) and in end-diastolic ventricular septum thickness [-0.3 (-0.2) mm], left ventricular posterior wall thickness [-0.2 (-0.1) mm], and a significant increase in systolic blood pressure (SBP) [2.5-3.5 (2.1) mmHg]. Moreover, OCT treatment was associated with a significant increase in SBP concentrations [P1] (i.e., 15 mg/kg) compared to placebo (15 mg/kg) and with a decrease in SBP concentrations [P1] (10 mg/kg) compared to placebo (15 mg/kg).

Conclusion: This meta-analysis provides a new window of cardioprotection during reperfusion. Among these, 120 were found to have occurred during recreational sport activities and 20 during professional sport-persons and in a rabbit model.

Introduction: We fused the C-terminal part of X-IAP (BIR3/RING) to the protein transduction domain (PTD) of HIV1 transactivator of transcription domain which carries Tat, a potent transmembrane protein of HIV-1, required for viral transduction. After anesthesia, 6–8 weeks-old C57/B16 mice were subjected to 30 min coronary artery occlusion followed by 24 h reperfusion (CAR). Intravenous injections of saline (control) or 0.8 μg/g of PTD-BIR3/RING were performed at 30 min, 1 h or 6 h after the onset of CAR. Caspase activities and the densities of several pro- and apoptotic proteins were assessed.

Results: BIR3/RING added 44 ± 2% 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 during reperfusion. These results also demonstrate that beyond the first minutes of reperfusion, a new window of cardioprotection exists within the first hours of reperfusion.
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Effect of age on treatment practices in patients with heart failure and preserved ejection fraction
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Introduction:
Heart failure with preserved ejection fraction (HFPEF) has replaced left ventricular ejection fraction (EF) being common in the elderly. Few intervention trials have been initiated in this specific population. One of the objectives of the ETICAS study was to evaluate the impact of left atrial discharge upon atrial fibrillation (AF) in patients with 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 77% at 1 year), 42 were 75 to 79 years (73 ± 5 years, 48% women, and 26% at 1 year), and 71 were 65 to 74 years (70 ± 7 years, 63% women, and 39% at 1 year). The mean EF was 63 ± 8%. The main aetologies in the two groups were hypertension (63%, 58%) followed by ischaemic heart disease (32, 26%). Medical management included diuretics and at 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 HFPEF. 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 antifailure daily doses at discharge or at 1 year, except for the ACE inhibitor and beta-blocker daily doses, which were lower at discharge in elderly patients. The present study emphasises the need for therapeutic studies in elderly patients with HF with 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). CHF 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 survivors in patient with advanced heart failure. Our secondary objective was to identify the prognostic value of MIBG scintigraphy in advanced heart failure and to correlate these results 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 over 10 years until death, hospitalization, or heart transplant. The comparaison of QTc values distributions of the different groups by using Mann–Whitney U-test.
Results: Patients with missing treatments information or ECG were excluded from postanalysis. Eleven patients treated with hydralazine 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).
Conclusion: Our results show that administration of hydralazine caused a statistically significant increase in QT interval measurements. By contrast, there was no significant relationships 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. This potential cardiac toxicity questions us on the capacity for the first-generation sedating antihistamines to expose to QT prolongation.

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Neuroprotective effect of sequential reperfusion: involvement of mitochondrial calcium channel (L-type), natriuretic peptide and neuroprotection induced by diazoxide
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Introduction: Cardiovascular baroreceptor control evolved early during evolution in order to maintain a fine adjustment of the cardiovascular system. The link between the central control of cardiovascular system and the neuroprotection was described by the central action of atrion giotensin II (ANG II) on the spontaneous baroreflex sensitivity (BRS) in our animal model, the unaesthetized trout. The central action of angiotensin II (ANG II) on the spontaneous baroreflex sensitivity (BRS) is reproduced by diazoxide and sodium nitroprussiate and reversed by 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. Patients were categorized into different groups based on the presence or not of hydralazine or alimemazine prescription. The aim of our study is to evaluate medical treatment at discharge and after 1 year in patients hospitalised for treatment. Eleven patients treated with hydralazine 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).
Conclusion: Our results show that administration of hydralazine caused a statistically significant increase in QT interval measurements. By contrast, there was no significant relationships 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. Multivariate analysis including the other risk factors for QT prolongation is needed to identify the patients at risk. In psychiatric practice, these findings could have important clinical relevance for patient taking concomitant drugs that can prolong the QT interval (e.g. hydralazine and tricyclic antidepressants) and those at risk of developing cardiac arrhythmias.

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Neuroprotective effect of sequential reperfusion: involvement of mitochondrial calcium channel (L-type), natriuretic peptide and neuroprotection induced by diazoxide
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Introduction: Potential cardiac toxicity of second-generation nonselective angiotensin II receptor antagonists has been largely becarefully examined during their development. It has been demonstrated that certain nonselective angiotensin II receptor antagonists have potential to prolong the QT interval, which predisposes to the development of Torsades de Pointes (TdP) and sudden deaths. First-generation sedating antihistamines such as hydroxyzine (diphenylmethane derivatives) and alimemazine and 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. Patients were categorized into different groups based on the presence or not of hydralazine or alimemazine prescription. The aim of our study is to evaluate medical treatment at discharge and after 1 year in patients hospitalised for treatment. Eleven patients treated with hydralazine 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).
Conclusion: Our results show that administration of hydralazine caused a statistically significant increase in QT interval measurements. By contrast, there was no significant relationships 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. Multivariate analysis including the other risk factors for QT prolongation is needed to identify the patients at risk. In psychiatric practice, these findings could have important clinical relevance for patient taking concomitant drugs that can prolong the QT interval (e.g. hydroxyzine and tricyclic antidepressants) and those at risk of developing cardiac arrhythmias.

Conclusion: Our results show that administration of hydralazine caused a statistically significant increase in QT interval measurements. By contrast, there was no significant relationships 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. Multivariate analysis including the other risk factors for QT prolongation is needed to identify the patients at risk. In psychiatric practice, these findings could have important clinical relevance for patient taking concomitant drugs that can prolong the QT interval (e.g. hydroxyzine and tricyclic antidepressants) and those at risk of developing cardiac arrhythmias.
Conclusion: The onset of the reperfusion is critical for pathway saliva 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 the targets list or merely to ameliorate mechanically the reperfusion manner in human.

131 Binding of elastin peptides to S-Gal protects the heart against ischemia/reperfusion injury in the RSK pathway
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Introduction: Degradation of elastin, the longest living protein, is a hallmark of atheromatous plaques and of several most cardiovascular diseases. As a consequence of such elastolysis, elastin fragments (EF) are released from the polymer and were detected in the blood circulation in a concentration range of 1 fg/ml. Level of EF increase significantly in the sera of patients suffering from abdominal aortic aneurysm, 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 (n=10) and then superfused for 40 min of global ischemia and 40 min of reperfusion (UK Ctrl); exposed to iPPs (1.12–660 nM) for 10 min at reperfusion. Hearts were treated with different inhibitors (U0126, L-NAME, Lactate and V14 peptide). 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: EF 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 effect. The EF-dependent effects were reproduced reproducibly, as well, and as being inhibited by receptor antagonists such as lactose and V14 peptide (VGVSPSAQEEAAL). EPs interaction with S-Gal triggered NO release and activation of PI3-Kinase/Akt and Erk 1,2 in human coronary endothelial cells (HCAECs and rat neonatal cardiomyocytes (RCC). This signalling pathway was 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 inhibitors. EP survival activity was attained at a concentration averaging that present into the blood circulation, supporting the content that there is a natural protector and as being inhibited by receptor antagonists such as lactose and V14 peptide (VGVSPSAQEEAAL). EF are released from the polymer and were detected in the blood circulation in a concentration range of 1 fg/ml. Level of EF increase significantly in the sera of patients suffering from abdominal aortic aneurysm, 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.

Conclusion: Our data support the contention that circulating elastin fragments or binding of elastin-derived peptides to S-Gal 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.

132 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 was shown to increase EPCs in peripheral blood. The purpose of this study was to determine the potential effect of chronic pravastatin therapy on early and outgrowth EPCs.

Methods: Two groups of patients were matched for sex, age and treatment. The statin (-) group (n=13) was composed of patients treated up to 4 weeks with 40 mg of pravastatin; (ii) long-term statin (+) group (n=13) was composed of patients treated with pravastatin 40 mg for at least 4 weeks. In both groups, patients were matched in terms of their cardiovascular risk factors.

Results: The mean number of circulating early EPCs were CD146+ and CD45+ and were also significantly higher in the statin (+) group, suggesting a beneficial effect of chronic statin therapy on early EPCs.

Conclusion: These data suggest a potential beneficial effect of chronic statin therapy on early and outgrowth EPCs in chronic CAD patients.

133 Critical role of angiotensin converting enzyme level in acute myocardial ischemia-reperfusion injury revealed by gene titration in the mouse
E Messiard-Ilari*#, R Zini*, R Soukla*, A Berdeaux*, D Morin* "Cirencester – France
Introduction: The peripheral benzodiazepine receptor is located on the outer membrane of mitochondria where it mediates the transport of cholesterol. This receptor is only clearly associated with the components of the mitochondrial permeability transition pore (mPTP) pathway but 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 structure and function, we studied the role of the peripheral benzodiazepine receptor in ischemia-reperfusion injury.

Methods: Anesthetized male Wistar rats were sacrificed to obtain cardiac tissue. The level of the receptor was measured by Western blot.

Results: Chlorodiazepam reduced infarct size expressed as the percentage of the risk area at 5 ± 1% (n = 6) and 10 mg/kg (11 ± 1% (n = 6) was compared with controls (31 ± 4% (n = 9)). This cardioprotective effect was associated with a reduction in apoptosis as demonstrated by a decrease in the number of tunel positive cardiomyocytes where the receptor was expressed.

Conclusion: This study demonstrates that the peripheral benzodiazepine receptor is a new target for protection of the myocardium against ischemia-reperfusion injuries.

134 Comparative study of the signaling pathways of the wild-type and deleted form of human z2B-adrenergic receptor, from the membrane to the nucleus
Introduction: A common polymorphic variant of the human z2B-adrenergic receptor (z2B-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 and protein-coupled receptor kinases. The variant form of the receptor is located at the outer mitochondrial membrane and as being inhibited by receptor antagonists such as lactose and V14 peptide (VGVSPSAQEEAAL). EF are released from the polymer and were detected in the blood circulation in a concentration range of 1 fg/ml. Level of EF increase significantly in the sera of patients suffering from abdominal aortic aneurysm, 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: Pig kidney LLC-PK1 cells were stably transfected to express similar levels of wild type (WT) and deleted form of receptor. Western blotting of phosphorylated Erk1/2, Akt and IKK/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 z2B-AR was decreased compared to that of the WT. Furthermore, depressed phosphorylation of the Del z2B-AR resulted in a prolonged coupling with G-proteins and a slower kinetics of β-arrestin recruitment.

Conclusion: The peripheral benzodiazepine receptor is a new target for protection of the myocardium against ischemia-reperfusion injuries.
positive staining cardiomyocytes [from 2.2 ± 9 to 6.6 ± 2%, (n = 6) P < 0.05]. Chlorotetrazolium showed the ability of mitochondria to synthesize ATP 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 chlorotetrazolium 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 evoked by their addition 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 injuries and that it reduced heart damage implicated 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, i.v.) or with only saline and subjected to laparotomy, liver 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°C in a preservation solution in a subculture of the presence of various BHDP concentrations (10 μM to 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 L-lactate and pyruvate into alanine and glutamine in the presence 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 functional 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 μM in the liquid of preservation during hypothermic ischemia. BHDP reduces 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 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**

**Introduction:** Rats reported here are the offspring of parental hypertensive rats and show hyperactive behavior characteristic of the SHR strain. Arginase 1 is an enzyme participating in the degradation of L-arginine, present in high levels in human and rat liver. Arginase is a key enzyme in the degradation of L-arginine and is involved in the synthesis of NO. The inhibition of arginase activity by the administration of L-NAME increases blood pressure and induces vascular remodeling.

**Methods:** Ten-week-old male SHR were treated with nor-NOHA (10 or 40 mg/kg, i.p.) for 3 weeks. One group of untreated SHR and normotensive Wistar Kyoto (WKY) rats served as controls. Systolic blood pressure (SBP) was measured in conscious rats by tail cuff method. The response of Ach was measured in aortic rings whereas the response of nor-NOH A was measured in mesenteric arteries.

**Results:** The good tolerance of arginase inhibition in SHR encourages to investigate the role of arginase inhibition in several models of hypertension. The effects of arginase inhibition were evaluated in SHR and compared to those observed in WKY rats. SHR was treated with nor-NOHA (10 or 40 mg/kg/d, i.p) for 3 weeks. Treatment with nor-NOHA dose-dependently elevated blood pressure in both SHR and WKY rats. Arginase inhibition did not affect the circulating levels of urea and glucose. 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 induce a decrease in M/L ratio.

**Conclusion:** In conclusion, ischaemic preconditioning (IPC) could be considered as a therapeutic strategy to protect the heart against the deleterious effects of ischemia-reperfusion. Moreover, IPC could limit the conversion of short-lived leukotriene mediators into long-lived leukotrienes, which may help to explain the beneficial effects of IPC.
141 The release of troponin I and the dynamic of circulating endothelial and hematopoietic progenitors in patients with acute coronary S Davant, F Deschaseaux, N Menreveu, JP Kantelip* Reanimation – France 

Introduction: Pericardial types of circulating progenitor cells can be detected in the peripheral blood of healthy subjects: hematopoietic progenitors (HPs), early and late endothelial progenitors (eEPCs). Early EPCs were described with a limited capacity to divide and to form endothelium (early and late EPC) and hematopoietic (CD45-CD144, GM and BFU-e) origin of the colonies were confirmed in vitro. The interaction of progenitor with myocardial infarction was also determined.

Methods: 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 (P = 0.03, R2 = 0.38). At day 7 there were no significant differences between the number of HPs and EPC's 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.

142 Effects of losartan in an experimental model of metabolic syndrome L Fellmann, P Bouquet * Strasbourg – France

Introduction: A large body of experimental and clinical evidence indicates that some AT1 receptor antagonists may have beneficial metabolic effects on their well-known cardiovascular actions. Whether or not these metabolic effects are related to additional PPARγ agonist activity of some AT1 antagonists is still under debate. 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 their cardiovascular and sympathetic nervous system is disturbed. 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 (P = 0.03, R2 = 0.38). At day 7 there were no significant differences between the number of HPs and EPC's and the rate of troponin release.

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143 Droperidol and ondansetron QT-interval prolongation: a clinical drug interaction study B Chabbi, JC Alvarez, JL Demole, C Funk-Brentano * Paris – France; 2Coches – France

Introduction: Droperidol and 5-HT3 antagonists, such as ondansetron, are the most commonly used 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 to the complete ban of this drug in France. 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 IV), ondansetron (OND 4 mg IV) alone and in combination. ECGs were recorded before digital image acquisition. Blood samples were obtained at the end of drug administration and after 2, 8, 45 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 the QTc. The mean (±SD) maximal QTc prolongation was 25 ± 11 ms after DRO alone and 20 ± 10 ms after OND alone. The combination of DRO and OND 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 AUC of drugs administered in combination/alone were 0.99 (IC90: 0.80 – 1.10) for DRO and 0.89 (IC90: 0.76 – 0.97) for OND.

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 antinemics does not additive or synergistic cardiac effects and is therefore not expected to increase the proarrhythmic potential of their combined use.

144 Genetic susceptibility to hypertension and chronic intermittent hypoxia as a rat model of obstructive sleep apnoea syndrome Belaïdi, B Lefebvre aa, F Stanke-Labesque a, G Dodin-Riboulot * Grenade – France

Introduction: Obstructive sleep apnea 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% FIO2) or normoxia (N), normoxia and IH, or IH alone, for 12 days. Body weight and systolic arterial pressure were recorded by plethysmography, were measured at days 1, 5, 14 (D1, D8, D15) of exposure. Blood pressure was also assessed at D15 following arterial catheterisation. The cardiac output was assessed at D15 by echocardiography. In order to assess infarct size following an ischemia-reperfusion (I/R) and at constant flow to study the coronary haemodynamic response to bolas injections of endothelin-1 (ET-1: 10–10 to 10–6 M).

Results: Body weight was greater in WKY than in SHR and in normoxics than in hypoxic animals. Hematocrit was significantly increased by hypoxia, particularly in the SHR group. The 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 ≤ 0.05). Catheterisation confirmed these results. IH aggravated infarct size both in 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 ≤ 0.05). In the SHR group, there was a significantly greater increase in coronary perfusion pressure (ΔP = 61.6 ± 5.7 mmHg) compared to normoxic animals (ΔP = 70.7 ± 4.9 mmHg and 83.3 ± 8.4 mmHg in SHR N, WKY III and WKY N groups, respectively, P ≤ 0.05) and in left ventricular systolic pressure in response to isoproterenol (ΔLPS = 10.3 ± 2.1 for IH and 11.0 ± 2.1 for N).

Conclusion: The combination of IH with genetic susceptibility to hypertension accelerates the development of hypertension, alters the metabolism and increases the risk of myocardial hypoxia. In conclusion, if IH is considered as 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.

145 Candesartan on the top of standard therapy decrease sympathetic activity in patients with advanced heart failure M Despas, E Guerriero, M Galinieri, JM Senard, A Pathak *Toulouse – France

Introduction: The CHARM trial has demonstrated that candesartan can significantly reduce all-cause mortality 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 random 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: 14 ± 6 ml/kg/M), the markers of oxidative stress were similar (MDM2: 3.95 ± 1.57 vs. 4.31 ± 1.07, P = 0.05), and there was no difference in ejection fraction and left ventricular size in the two groups (P = 0.05). 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.

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 and hormonal system or effect of candesartan on Hemoglobin, a well known prognostic factor in heart failure.

146 Temporal and spatial changes in free iron levels after brain ischemia in mice F Millerot-Serrurero*, C Mossier, C Marie* Dijon – France

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

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Methods: A cortical infarct was induced in rats using the photothrombotic ischemic stroke model. Measurements of ultralow iron (atomic absorption spectrometry) and PT (Western blotting) were performed in control and ischemic rats. Cortical tissue samples corresponding to the ischemic core (IC) and penumbra (PC) were collected at time 1 h post-ischemia (57.6 ± 1.2 vs. 176.4 ± 46.2 for IC and 55.7 ± 55.5 at 155.9 ± 29.9 for PC). Consistent with the rapid return to normal values of free iron level, DP did not reduce iron expression, whereas those to angiotensin I were unchanged. At 151 g/kg/min this effect was modest and not significant vs. control (Table 1).

Conclusion: Our results report different spatial and temporal profile of expression between ischemia-reperfusion injury and control brain ischemia in rats. Expression of PT and control administration just before and after brain ischemia was already reported to reduce BBB damage. Finally, ischemia resulted in a delayed and sustained FT upregulation after brain ischemia. They suggest that the effects of hypoxia on the angiogenic properties of EPCs viability and angiogenic potential dramatically depend on FT expression modality.

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Characterization of the meta-nitrobiphenyle carboxamides as new 2-adrenoceptor subtype agonists

Introduction: The imidazoline derivative biphenyline displays an interesting potential for the treatment of many diseases including gastric ulcer, intestinal problems, hyperglycemia and metabolic syndromes. The imidazoline receptor is a G-protein coupled receptor which has been linked to the modulation of hypoxia-induced angiogenesis by upregulating KDR in vitro and in vivo. We investigated the effects of bis-(1-methyl-3-phenyl-1H-imidazol-2-yl)ethane (mNBP) on hypoxia-induced angiogenesis. 

Methods: Methods: mNBP was used to treat four human endothelial cell lines (in vitro) and in an orthotopic model of hypoxia-induced angiogenesis (in vivo) 

Results: mNBP inhibited hypoxia-induced angiogenesis. In vitro, mNBP inhibited hypoxia-induced cell proliferation, increased the expression of the hypoxia marker carbonic anhydrase 9 (CA9) and decreased the expression of the hypoxia marker angiomotin in the two human endothelial cell lines tested. In vivo, mNBP reduced the size of the new vessels formed in the tumor sphere associated with a significant decrease in the expression of the KDR receptor.

Conclusion: mNBP is a potent inhibitor of hypoxia-induced angiogenesis and could be a new drug candidate for the treatment of hypoxic diseases such as cancer.

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Hypoxia differentially regulates angiogenic properties of human endothelial progenitor cell cultures
O. Lebrun,a D. Galliard,b I. Codacciob, F. Saliier,b F. Diguet-Georgena, P. Pinsana, Marseille – France

Introduction: An increasing number of studies provide evidence that hypoxia might promote cell survival and enhance neovascularization efficacy. We evaluated the effects of hypoxia on human endothelial progenitor cell (EPCs) and their potential therapeutic applications.

Methods: HUVECs were cultured in hypoxia for 24 h in growth factor-free medium with 5% of fetal bovine serum, in presence of cobalt chloride (CoCl2, 150 μM: chemical hypoxia) or in oxygen free-atmosphere with bubbled-medium (5%CO2, 10%H2, N2: drastic hypoxia) or in 2%O2 (5%N2, 1%CO2, 94%H2) for the treatment of many diseases including gastric ulcer, intestinal problems, hyperglycemia and metabolic syndromes. The imidazoline receptor is a G-protein coupled receptor which has been linked to the modulation of hypoxia-induced angiogenesis by upregulating KDR in vitro and in vivo. We investigated the effects of bis-(1-methyl-3-phenyl-1H-imidazol-2-yl)ethane (mNBP) on hypoxia-induced angiogenesis. 

Results: FT 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 altered by chemical (MTT: 82 ± 7% and LDH: 8 ± 5%) and drastic hypoxia (MTT: 84 ± 7% and LDH: 23 ± 6%) treatment. FT expression was significantly enhanced, whereas those to angiotensin I were unchanged. At 151 g/kg/min this effect was modest and not significant vs. control (Table 1).

Conclusion: Our results report different spatial and temporal profile of expression between ischemia-reperfusion injury and control brain ischemia in rats. Expression of PT and control administration just before and after brain ischemia was already reported to reduce BBB damage. Finally, ischemia resulted in a delayed and sustained FT upregulation after brain ischemia. They suggest that the effects of hypoxia on the angiogenic properties of EPCs viability and angiogenic potential dramatically depend on FT expression modality.

150

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Conclusion: mNBP is a potent inhibitor of hypoxia-induced angiogenesis and could be a new drug candidate for the treatment of hypoxic diseases such as cancer.
evaluate the hypotensive activity of the aqueous and methanol extracts from S. torvum fruits. The anti-aggregatory 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 was evaluated in anaesthetized rats using the invasive method. The rats were divided into groups: control, rats treated with a saline solution (control), 100 and 200 mg/kg of genistein (Geni100 and Geni200, respectively), and 10 and 100 mg/kg of 17β-estradiol (17βE10 and 17βE100, respectively). We used a low dose of genistein to avoid tyrosine kinases inhibition. The crude extract at the doses of 1 and 2 mg/kg reduced blood pressure without affecting the heart rate. However, no direct evidence has been documented so far. In this work, we used in vitro experiments to characterize the anti-oxidant and anti-inflammatory effects of the extracts. The anti-aggregatory activity of the crude extract may be beneficial for the cardiovascular system. This effect is mediated by the peroxynitrite antioxidant activity of the aqueous extract. Meanwhile, yohimbine almost completely inhibited the cardiac effect of the extract at the dose of 5 mg/kg. When assessed 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 dependent manner the aggregation induced by thrombin or Adenosine diphosphate.

**Conclusion:** The present study suggests that the aqueous extract of S. torvum possesses hypotensive activity which may result at least partially from its bradycardic 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 suggested that this extract might not affect the cardiovascular system. The anti-aggregatory activity of the aqueous extract may be beneficial for the cardiovascular system. The fact that this anti-aggregatory activity does not depend to the aggregated agent suggested that the extract may be acting on the step of aggregation common to both agent.

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

**A. Vergeade, P. Guisset, M. Isabelle, C. Thuillez**

**Rosen – France**

**Introduction:** Cocaine use involves many adverse effects on the cardiovascular system. Cocaine use is associated with ischemia, arrhythmia, 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. We will investigate the subcellular site involved in this production.

**Methods:** Wistar rats were treated with saline solution (control) or cocaine hydrochloride (24.7 ± 0.5 mg/kg/day IP) for 7 days. Cardiac function was evaluated by echocardiography. ROS production was measured by EPR spectroscopy in conjunction with the spin probe 1-hydroxy-1-methoxy-2,2,5,5-tetramethylpyrroline (CMH), with or without mercaptopropylguanidine, a peroxynitrite neutralizer. These evaluations were made in LV homogenates and in subcellular fractions of mitochondrial subsarcomembran mitochondrial (SSM) and interfibrillar mitochondria (IFM) in absence and in presence of substrates to initiate respiration.

**Results:** 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 CMH formation. Addition of MEG in the assay almost completely abolished the spectra indicating that formation of CMH is in part mediated by the peroxynitrite. In subcellular fractions, substrates-fueled mitochondria generated free radicals 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 peroxynitrite production. Moreover, this study also suggests a critical role for IFM as an important site of peroxynitrite production. The crude extract at the doses of 1 and 2 mg/mL was essayed on platelet aggregation induced either by thrombin or Adenosine diphosphate. Only the concentrations of 1 and 2 mg/mL were effective on platelet aggregation, induced by either thrombin or Adenosine diphosphate.

**Conclusion:** Besides the anti-aggregatory effect of cocaine, 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.
out of notification data, patient pharmacopoeia behaviour by calculating a seriousness score. The more frequently predisposed potentially serious adverse event was,
the current status of the validation process which consist in benchmarking
pharmacopoeia seriousness of targeted drugs, against substances whose dependencies
have been validated.

Methods: The method consists of a questionnaire which includes (i) a series
of questions derived from the items of BMV-IV of the definition of pharmacoden-
penicillin, (ii) a list of expected values of the specific tool and (iii) a notation
aimed at evaluating patient transgression behaviours (fraud and misuse). The
answers are then processed to calculate a score of pharmacopoeia seriousness
for each substance targeted in the notification. 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 that contribute to the approval process of the method. As French CEIP receive all
notifications from CEIP's, represents a such powerful combination that all French CEIP's
are considered to be involved.

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
will benefit from the method. On average, the method analyzed together about 1800 notifications each year. Nantes CEIP will soon have a very
valuable material to validate its novel tool.

157 Re-evaluation of drug treatment in the elderly: is it necessary and possible ?
J Doucet, Th Trinh-Duc, P Moirat, B Lefevre, M Mercier, Rouen - France; Agen - France
Introduction: Polypharmacy and increased risk for adverse events are
frequent among elderly patients. To encourage to regularly re-evaluate drug therapies 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 include 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 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 32.5% of ‘cardio-vascular’ drugs, 40.1% of ‘neuro-psychiatric’ drugs, 53.7% 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 previously treated, new indication or disappeared indication (26.6%), therapeutic optimization (17.1%), adverse event (6.1%), not proved efficacy (4.8%), high risk for adverse event (3.5%). But the frequencies of reasons for modifications differed between the different drug
classes.

Conclusion: The re-evaluation of drug administered in chronic disease in the elderly
may be of interest to improve more therapeutic practice. It was performed before and 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.

158 Quality of life questionnaires in skin diseases: use in randomized clinical
trials
LJ Clech*, O Chassany*, A Levy*, P Wolkerstein*, O Cloidow* 4Corbel – France; Paris – France; Cretéil – France
Introduction: The European Regulation on Quality of Life Assessment (ERQA) working group published recommendations on the use of QOL questionnaires as an evaluation endpoint in randomized clinical trials (RCTs). The objective of the systematic review was to gather the reports on RCTs measuring QOL in dermatology, and analyze them based on the ERQA 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 1986 to February 2005. The studies using at least 1 QOL questionnaire to measure skin diseases in English or French were selected. We excluded studies concerning cancers, and human immunodeficiency virus (HIV). We analyzed each retained RCT according to the ERQA checklist.

Results: Out of 574 titles (Medline), 267 (57%) were excluded based on their abstracts, because of search overlap (n = 305), other language (n = 2), and absence of clinical research (n = 4). Out of 122 RCTs, 93 (77%) were excluded after full review: no QOL (n = 13), QOL evaluation (n = 32), QOL questionnaire (n = 65). The reduced sample of 29 articles was analyzed. The first analyzed study was published in 1993. 77% (11) of the analyzed studies were published between 2001 et 2004.

Twenty distinct QOL questionnaires were used. The Dermatology Life Quality Index (DLQI) used by 37% (16) was the most used. The second one was the 32% (14) of the 43 RCT were double-blind. QOL was the a priori judgment criterion in six studies. 12% (5) of the retained trials justified the choice of a general or specific questionnaire. Nineteen percent of the reports described the use of several QOL questionnaires. 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 3% (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. Most reports were written in English. 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.

159 Local survey of long-acting injectable risperidone prescription practices E Morice*, C Guibaud*, C Gabriel-Bordenave*, V Auclair, C Robege* 4Carn – France
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, on 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, Insern and university research units. The CIC/P network contributes to technical innovations, has facilities designed for the conduct of research, and provides pharmacovigilance. They are subject to high quality research and children are not subjected to unnecessary clinical trials.

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 and paediatric surgery.

Material and methods: The Paediatric CIC network already provides pharmaceutical industries with competences in paediatric research and facilities specially designed for the conduct of clinical trials in children. The CIC/P network is a 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.

160 The Paediatric CIC network - Year 2006 E Jacqz-Aigrain*, B Kassa*, and the members of the French CIC network *Paris – France; Lyon – France
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161 Beneficial hemodynamic effects of activated protein C in a rodent model of endotoxic shock R Favory*, S Lansel*, R Nevire*, D Durrocher* 4Lille – France
Introduction: Cardiovascular failure is one of the determinants of mortality in the context of septic shock. Our question was: Does activated protein C (APC) have positive hemodynamic effects in a rodent model of endotoxic shock?

Methods and materials: Male Sprague-Dawley rats (250 - 275 g). Internal jugular vein and carotid artery were tunnelled subcutaneously for arterial pressure measurement and drugs administration. Four groups were studied: controls; physiological saline; 20 U/mL and 40 U/mL APC.

Results: APC infusion in control rats induced a significant decrease of mean arterial pressure (22.4 ± 2.1% 20 U/mL and 29.3 ± 1.8% 40 U/mL) and increases of heart rate (11.4 ± 2.4% 20 U/mL and 21.1 ± 2.9% 40 U/mL). APC infusion in APC-infused rats induced a less significant decrease of mean arterial pressure (17.6 ± 1.1% 20 U/mL and 21.1 ± 1.8% 40 U/mL) and increases of heart rate (11.1 ± 1% 20 U/mL and 20.5 ± 1.2% 40 U/mL).

Conclusion: APC infusion in APC-infused rats induced increases of heart rate and mean arterial pressure compared to controls.
Controls + APC 240 µg/kg/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 + 240 µg/kg/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 comparisons with controls.

Results: In the LPS group there is a drop in mean arterial pressure over the duration of the 4 hours. Arterial LPS-induced drops in arterial pressure over time 107 ± 7 mmHg vs. 82 ± 6 mmHg (P < 0.05).

APC preserved arterial pressure and contractility decrease at 1h/14YDP 8 ± 4 mmHg for APC treated and 60 ± 5 mmHg for LPS (P < 0.05), dP/dtmax 3100 ± 90 mmHg/s for APC treated and 2700 ± 80 for LPS (P < 0.05). The dose-related suppression of arterial pressure increase function of dose of norepinephrine was flat for the LPS group. A complete recovery was seen in APC treated animals. 55 ± 6% of delta arterial pressure for APC treated rats compared with 10 ± 7% for LPS (P < 0.05). TNFLA is less increased in APC group (130 ± 23 ng/mL vs. 230 ± 30), plasmanatrates/nitrates too (6.5 ± 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, nitrate/nitrites, MIF.

164 Rifamycin lavage in the treatment of experimental intra-abdominal infection
Introduction: To investigate the effect of peritoneal lavage with rifamycin to reduce the number of intra-abdominal 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 24h, 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: saline 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 during the 7 days were necropsied. 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 < 0.05.

Conclusion: The results suggest that peritoneal lavage with rifamycin reduces mortality, the number of adhesion and the bacterial counts and might be useful in the treatment of IAI.

165 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
Introduction: Fish oil-derived n-3 PUFAs have prompted a growing interest in chronic inflammatory states, such as Crohn’s disease (CD). Indeed, N-3 PUFAs are known to inhibit the production of pro-inflammatory cytokines and to decrease the production of pro-inflammatory cytokines.

Methods: PBMCs have been obtained from peripheral blood from fasting Crohn’s disease patients and healthy controls. n-6 PUFAs (linoleic acid) were replaced by 37% [humidified air (95%) / CO2 (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 3300, Endolipide or Endolipide a.

Twenty-four hours of culture, supernatants were removed and stored at -80°C until cytokine measurement by ELISA (R&D Systems). TNF-alpha serum levels were measured by a competitive ELISA.

Conclusion: These results suggest that peritoneal lavage with rifamycin reduces mortality, the number of adhesion and the bacterial counts and might be useful in the treatment of IAI.

166 Comparison of France vs. the rest of the World on use of venous thromboembolism prophylaxis in patients hospitalized for acute medical conditions: a prospective registry
Introduction: Although it is generally accepted that thromboprophylaxis reduced mortality in patients hospitalized for acute medical illness, an observational cohort with multinational sites is of importance to assess the thromboprophylaxis use in various geographic areas.

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.

Comparison: Lamivudine remained effective in HIV- HBV co-infected patients, but some groups of patients (6%) with persistently elevated transaminases or flare hepatitis had 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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Elevated levels 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’) in the medical domain 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 ‘active’ 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 included: absolute mean variation of score of real prescriptions that were consistent with the simulated cases.

Results: Twenty-two French units (departments) of cardiology were randomized (2:1 active transfer group) and 28 cardiologists from 17 departments were included. The adjusted absolute mean improvement of score was more improved than in the control (P= 0.031 at the department level, absolute mean absolute improvement of live points/100). The change in knowledge transfer (MO) was significantly different between the two outcomes. However, no benefit was shown in terms of prescription conformity to evidence. For the ‘passive’ mode of knowledge transfer and for the three outcomes, no differences were apparent between the two tested modes 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 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. H2-receptor antagonist (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 patients following major surgery.

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. We performed a random effect model (Review Manager 4.1). 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, primary outcome (bleeding requiring endoscopy or blood transfusion), postoperative mortality (POM), and mortality (M) (secondary outcomes). Study heterogeneity was sought and tested interventions: absolute mean variation of score (difference between post and pre study session) of knowledge transfer, and a hierarchical structure (cluster of physicians for each department level).

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=1), 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, P2 = 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 = 0.035 P2 = 66.7%, I2 = 55.5%, OR = 1.35, (0.82; 2.22)], for which there was no observed heterogeneity (P = 0.95, P2 = 0%). Interestingly, although all studies showed lower rates of bleeding for PPIs vs. H2RAs, only one study with bleeding [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, PPIs did not significantly reduce the 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 PPIs. 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 a homeopathic treatment on cumulative faecal incontinence rate delivered by PCP (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 control group (homeopathic placebo), and two intervention groups (homeopathic pramipexole and ropinirole) and an open-label non-interventional 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 (‡) 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.

Conclusion: The questions examine also the neurologic and orthopedic 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 information who explain the many complications of the surgery (pneumonia, renal insufficiency leading to the dialysis, sixty-three per cent of the patients have a feusal incontinence often associated with chronic constipation (94% of patients)), the gastrointestinal transit (which is only 13/165 have undergone a proctologic exploration. The sexuality is accomplished with difficulties: only 31% of the ladies and 53% of the men have sexual intercourse; the disorders of the children’s sexual education is the more frequent problem (51% of 11% of the ladies) with healthy children.

Conclusion: A regular following up is needed in all the concerned medical disciplines (and not only urologic, gastroenterologic, sexologic but also neurologic, orthopedic and physiotherapy) will permit the spina bifida adults patients (about 20 000 patients) to have a better quality of life, to avoid the most serious complications (renal insufficiency and neurologic complications with pain) and the daily difficulties bound to incontinence, constipation and sexual intercourse. This following up would have to be assured by regional and multidisciplinary clinics with a seal of quality, therefore labelled.

174 Incontinence and insufficiency of following up and medical care of patients with spina bifida

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Introduction: A questionnaire specially directed towards the problems of incontinence and insufficiency of the follow-up 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 insufficiency of the follow-up of patients in order to precise their difficulties and to bring on responses to their needs.

Conclusion: The results of this meta-analysis showed with a probability of 99.9% that PPX was superior to RPR with a probability of superiority of 99%, 98% and 95% respectively.

175 Meta-analysis on survival data comparing docetaxel and vinca alkaloid 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. standard chemotherapy. An initial search identified 32 RCTs; 14 were excluded on the basis of 1 or more of the following criteria: duplication (n = 3), not a first-line treatment of advanced NSCLC (n = 3), not a randomised controlled trial (n = 5), non-comparative design (n = 5), and no survival data (n = 7). The remaining total of 18 trials were included in the meta-analysis. The results of this meta-analysis support the positive benefit-risk ratio of docetaxel compared to vindesine or vinorelbine-based chemotherapy in the first-line treatment of advanced NSCLC.
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 unpopular 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 tolerability of a vaccine. The follow-up period was 59.9 ± 3.15 days. Treatments with immunomodulating agents were excluded, and the patients were principally men (76%) while sex was equally distributed 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 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 BP and possible cognitive dysfunction.

Methods: The OSCAR study is an international longitudinal observational study with a duration of 6 months intended to examine the impact of eprosartan on cognition compared with the Mini-Mental State Exam (MMSE) and on control of systolic blood pressure (SBP) in a large international population of hypertensive patients managed in a standard primary care setting. A total of 108 centers were included, 52% of patients (n>200) MMSE < 26. OSCAR 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 administrated before the beginning of the treatment. All patients will receive a dose 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 12 397 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 function and 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.

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 studied. Primary study outcome measures were 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 or death. Central independence and further analyses. No significant differences in acute rejection episodes, dialysis occurrence or death were observed between groups (P>0.05). However, a trend toward a lower increase was found in favour of LT treatment. These trends were observed in average with a strong individual variability and should be confirmed with further studies. In conclusion, a acute rejection episodes, dialysis occurrence or death were observed between groups. Rejections occurred in 51% and 46% of patients in respectively LT and SD. One patient from the LT group and one patient from the SD group died. One patient from the LT group and two patients from the SD group died. One patient from the LT group and three patients from the SD group died.

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.
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 studies were contacted and requested to submit unreported references lists and abstracts of congress were hand-searched.

Selection criteria: All randomised controlled trials comparing induction therapy with placebo or other induction therapies were included. Any kind of randomization and effect model will be used to summarize relative risk for dichotomous outcomes with their 95% confidence intervals (CI).

Results: A total of 2036 articles and 55 abstracts of congress were identified. After screening of titles and abstracts, 1529 papers were excluded. We performed a full paper review on 507 articles. Altogether, a total of 141 articles corresponded to the inclusion criteria (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 BMA/051.

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-jection (panel reactice antibodies positivity, HLA mismatch, cold ischaemia 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 (Beech-Bed) which allows to be used for positioning cushion 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 covering age ranges: 2-6 years, 6-10 years, 10-15 years, 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/mm³). Secondary outcomes included: child’s pain using a visual analog 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 this 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% for success and 15% vs. 24% for post LP syndrome) in children in pain (VAS ≥ 50 mm, 7 children) and less in pain children (VAS ≤ 50 mm, 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 positioning cushion cushions 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 strategies. The 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.

Methods: To realistically simulate the evolution of the disease, the nature of the disease 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 in other organ systems, the capability of regaining systemic homeostasis decreases dramatically within only a few hours.

Methods: Taking into account the complexity that lies beneath this pathologic process, we have set up a multidisciplinary team composed of physicians, mathematicians, immunologists and pharmacologists. Gathering and assessing the third 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 in France, a cohort of 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 further validated 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 the model.

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.
reduce inflammation by direct pro-inflammatory effect and increase anti-oxidant cell capacities by a selective incorporation into mitochondria. 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 vasoressor 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 vasoressor therapy withdrawal was 7 days in both groups [95% CI = (5.8–5.1)] (6–9) in placebo and selenium groups, respectively; log-rank: P = 0.171). The duration of mechanical ventilation was 25 ± 41 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: 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.
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$_{24h}$ (Concentration controlled’ C 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 prospectively by a research team and provided to a 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 all non covered extra costs (MTP), mainly transport costs. Incremental analysis was performed by comparing ratios of the difference in costs to the difference in one or the other effectiveness criteria between the C group and the FD group. Cost to avoid acute rejection, one acute rejection episode with MMF (TDM) were calculated.

**Results:** The incidence of treatment failures was to 29.2% (C group) vs. 47.7% (FD group) (P = 0.03). This difference was mainly due to a difference in clinical acute rejections (12.3% vs. 30.8%, P = 0.01), while the incidence of ADE was slightly but not significantly higher in the C group (97% vs. 91%, P = 0.3). The cost per treatment failure was 665 euros (C group) vs. 84942 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.

**Conclusion:** 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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192 Therapeutic drug monitoring of tipranavir: about two cases

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**Introduction:** Tipranavir is a nonpeptide protease inhibitor (NPI). A resistance profile distinct from that of other currently available protease inhibitors (PIs) has been observed in vivo and in vitro. Resistance of PIs to tipranavir increases tipranavir plasma concentrations: therefore, the approved dose of tipranavir is 500 mg with ritonavir 200 mg, twice daily (1). Severe adverse effects of toxicity (lipodystrophy and lipid abnormalities) require 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 assess therapeutic drug monitoring of tipranavir, we validated an HPLC method 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 high performance liquid chromatography (HPLC) quantification and validated its use to determine simultaneously plasma levels of 11 molecules in a single run.

Pre-treatment of 1 mL of plasma sample spiked with an internal standard (in-house synthesis) was made by a solid phase cartridge (Waters Oasis HLB) using an ASPEC® automatic (GILSON®). This system allows injecting directly samples on line, without further step of experiments. Liquid chromatography was performed using a Symmetry C18 column (150 mm × 4.6 mm, 5 μm) 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 1000 ng/mL and 80% for the other drugs. Coefficients of variation were lower than 15% for all drugs. We confirmed by assays that patients treated by tipranavir/ritonavir (500/200) mg twice daily with hepatic disorders (elevation of ALT/AST and hepatitis) presented overdosage of tipranavir (respectively 11 007 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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193 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 eflux transporters, such as P-glycoprotein (P-gp), which transports drugs out of intestinal cells and reduces net absorption of drugs. In the everted gut, the everted part is exposed to normal bile salts 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 in saline, and finally saline and insulin solution (50 g/L) was incubated in vitro at 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, therefore, reliable monitoring assays and adequate ciclosporin A concentrations are crucial. Historically, methods to monitor blood levels of ciclosporin A can be divided into high performance liquid chromatography assays and immunosassays. These assays differ in their specificity, precision and their analytical performance characteristics.

The aim of this study was prospective, it is to compare the new Abbott AxSYM® ciclosporin assay with high performance liquid chromatography (HPLC). This system allows injecting directly samples on line, without further step of experiments. Liquid chromatography was performed using a Symmetry C18 column (150 mm × 4.6 mm, 5 μm) 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 1000 ng/mL and 80% for the other drugs. Coefficients of variation were lower than 15% for all drugs. We confirmed by assays that patients treated by tipranavir/ritonavir (500/200) mg twice daily with hepatic disorders (elevation of ALT/AST and hepatitis) presented overdosage of tipranavir (respectively 11 007 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.
197 Population pharmacokinetic analysis of ultrafilterable ciplatin after peroperative intraperitoneal administration
B Royer\textsuperscript{a}, V Julien\textsuperscript{a}, E Guardiola\textsuperscript{a}, D Montange\textsuperscript{a}, G Holey\textsuperscript{a}, D Delroëux\textsuperscript{b}, B Chaussain\textsuperscript{a}, H Segard\textsuperscript{a}, JP Kuntz\textsuperscript{a}, Beaumon - France; \textsuperscript{b}Paris - France; \textsuperscript{c}Reims - France; \textsuperscript{d}Dijon - France

Introduction: Ovarian cancer is the leading cause of gynecological cancer-related death in women. Peroperative intraperitoneal chemotherapy (IPC) is one of the most promising treatments currently available on intraperitoneal chemotherapy. Using such method, we previously described pharmacokinetic parameters of platinum with a 2-h intraperitoneal IP treatment and established that IP CIP concentration revealed for cytotoxicity was insufficient. Here are presented a population pharmacokinetic analysis obtained after two consecutive one-hour IP administrations of 30 mg/L of Ciplatin.

Methods: Twenty-eight patients with advanced epithelial cancer classified FIGO stage IIIC underwent a debulking surgery during which the intraoperative intraperitoneal chemotherapy was delivered by Ciplatin (240 mg/L per 30 min/hour-bath with 30 mg/L of Ciplatin). 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 KF-M8. The population pharmacokinetic study was conducted on the nonlinear mixed effects 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 fit 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 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\textsuperscript{-1}. Among the covariables tested (age, weight, body surface area, height, serum creatinine, creatinine clearance, serum total protein and albumin). 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 demonstrates the feasibility 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 sample strategy for the determination of patient’s exposure.

198 Combined inhibition of monoamine oxidase and semicarbazide-sensitive aminooxidase (SSAO) in the enterohepatic cycle in obese rats
C Carpine, Z Soltesz, D Previé, S Bour, P Valet, Toulouse – France

Introduction: Aminoguan, 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 adipoocytes 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 adipsic tissue deposition in the hyperinsulinaemic obese Zucker rat.

Methods: Aminoguanidine was i.p. administered at 270 mg/kg/day for 3 weeks to anesthetized Zucker rats. The other rats received 100 mg/kg/day of pargyline alone or in combination with pargyline (P, 20 or 61 mg/kg/day) for 3 to 4 weeks. Results: Aminoguanidine-treated rats lost their SSAO activity in adipose tissues and cerebral vessels (32%), as well as in total 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 SSAO and MAO in all tissues. Although without clinical effects, pargyline was 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. Adipoocytes from P+S-treated rats responded to insulin activation of glucose uptake at control but became unresponsive to the insulin-like actions of benzylic (SSAO substrate) or tyramine (MAO substrate) plus vanadate, while those from AG-treated rats lost only their response to benzylic.

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

199 Effect of CYP2C19 polymorphism on nelfinavir to M8 biotransformation
D Hirt, F Mentre, A Tran, E Rey, S Auleley, X Duval, D Salmon, JM Treluyer - Paris - France

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

Methods: Blood has been collected from 64 healthy volunteers. Blood samples (191 M8 concentrations were measured in 34 naïve-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 were stratified in 3 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 to 24 h. The coefficient of determination was calculated by the Collonformula: Sc = 2 Cuf/(Ca + Cv).

Results: First, we have found that the elimination of the VRC in CHIV 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 CHIV, the percentage of VRC elimination remains unchanged before or after a prolonged extra-corporeal circulation. But in post-dilution CHIV, this percentage is significantly increased above 40%.

201 Acute intoxication by tramadol: interest of pharmacokinetics follow up during the critical phase of survival
V Magnaud, V Leong-Boulenger, JP Goulle\textsuperscript{a}, C Lacroix\textsuperscript{a}, D Debruyne\textsuperscript{a}, A Coquerel\textsuperscript{a} Courn – France; \textsuperscript{b}Le Havre – France

Introduction: Tramadol (TMD) is a synthetic opioid used per os. In WHO classification it is a group Ia analgetic. 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 K\textsubscript{i} are 2.4 and 0.62 nm, respectively); one of its metabolites [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: 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 (RECA). At admission Glasgow was three and the tachycardie became asystolie. Fortunately the patient completely recovered during the critical phase of cardiac sideration. Fortunately the patient completely recovered.

Methods: DMT and ODT where measured in blood after admission (corresponding to 10th hour post intoxication), 12 h later and every day until day 8. D8-MLS/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 during the 24th h. Then we observed T1/2 of 19 and 23 h for TMD and ODT respectively during the 6 days of ECCA. Finally the patient became normal at 12 days. But in post-dilution CHIV, this percentage is significantly increased above 40%.
In vitro models to complete hERG channel assay in preclinical cardiac safety pharmacological studies

J Ducroc, P Printémpt, S Guibout, C Salvatier, M Le Grand Lanthier – France

Introduction: For the prediction of the hERG channel block of clinical antiarrhythmic compounds, the demonstration 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 cannot 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 NaV1.5 and hERG currents, on the action potential parameters in rabbit atria and atrial 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 μM) and flecainide (10 μM) have been first investigated on hERG and NaV1.5 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 (APD90) +15% and +69% at 1 Hz and in action potential triangulation (APD10-90) +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 12% NaV1.5 inhibition induced by quinidine led to a decrease in depolarization rate (dV/dt up) more marked in atrial action potential (-20%) compared to Purkinje fibres (-13%) at 1 Hz. In the same way, flecainide blocked atrial dV/dt up by 75% 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 already 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 an appropriate model for detecting compounds with 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.

203 Compliance of slow acting drugs in osteoarthritis in medical general practice

L Dejilez – Paris – France

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 by comparing them with non-drugs coils in different randomized controlled trials.

Methods: National transverse descriptive epidemiologic investigation in general medical practice: 991 general practitioners described 991 ambulatory patients suffering from osteoarthritis and were asked to mention 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 medium duration (42.7%). It is associated with oral Nsaid drugs in 32% of the cases and analgesics of level 1 in 57.5% of the cases. The duration of the treatment is higher than 1 months in 42% of the cases, continuously (52.2%), by cure applying at a rate of 2.6 cures/year. It is continued between 2 and 5 years in 45.4% of the cases.

Functional reimbursement is associated with pharmacological treatments in 78.5% of the cases and with other nonpharmacological treatments in 60.5% of the cases.

The observance of the treatment by slow acting drugs in osteoarthritis and the analigic 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).

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 habitually 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 these patients.

The conclusion: The observance of the treatment by slow acting drugs in osteoarthritis and the analigic treatments is considered to be good or good enough mainly for 81% of the general practitioners. That of local NSAID and 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.
Measurements of tail skin temperature in rats: a model of hot flushes

E Bracy,* A Maurin,* P Champeaux,* S Richard* Baugy – France

Introduction: Menopause is associated with the increase in skin temperature, 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: Tail skin temperature and locomotor activity were recorded using telemetry 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 mg/kg weekly injections instead of every 2 weeks in patients with renal insufficiency underlying menopause. Administration may be performed anywhere before or after the session on hemodialysis days.

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 underlying menopause. Administration may be performed anywhere before or after the session on hemodialysis days.

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An enzyme-linked immunosorbent assay to study bevacizumab pharmacokinetics

T Derrant,1 T Lecomte,2 D Degeenne,3 AC Duveaux,4 H Watier,5 G Paintrand Tours – France

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 microtitre 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 (Bio-Rad). Absorbance was measured using as 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 above the mean of five replicates.

Results: LOD was 0.013 µg/mL. The intraday precision indices of the method were (per cent coefficients of variation): CV(a) 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 was (per cent deviation) were +4.1%, -5.9% and +2.1%, respectively. Lower limit of quantification (LLOQ) and upper limit of quantification (ULOQ) were therefore 0.006 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 = 3.15 (L2.2%), CL = 3.86 (L1.4%), Vp = 2.01 (L1.14%), CLp = 0.05 (L1.9%), T1/2 = 0.6 (L18%) and T50% = 21 days (L16%).

Conclusion: We have developed a reproducible method for measuring serum bevacizumab concentrations in treated patients with a standard deviation from 3.06 to 74.8 µg/mL. These preliminary results suggest a high interindividual pharmacokinetic variability in colon cancer patients.

Critical evaluation of the ferret emesis model in safety and efficacy pharmacology studies

S Picard, S Goineau, P Guillaume, P Lacroix Toulouse – France; Toulouse – France

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 who live or work with nicotine and cotinine, primary metabolite of nicotine after CO-oxidation by the cytochrome P450, have been widely used as biomarkers to determine cigarette smoking status and passive exposure to environmental tobacco smoke. In the French National Study on the role of passive and active tobacco exposure, we investigated the use of hair samples for quantifying nicotine and cotinine. This high performance liquid chromatography and capillary electrophoresis, and cotinine total nicotine metabolites by colorimetry.

Results: Hair was taken from 15 non smokers and 15 smokers. This hair was incubated in a NaOH solution at 100°C for 1h. The samples were then extracted using diethyl ether. Nicotine content was evaluated using gas and liquid chromatography and capillary electrophoresis. We measured nicotine total metabolites levels (in cotinine-equivalents) measurements.

Conclusion: Active cigarette smoking exacts a continuing toll on public health, it is an established risk factor for cancer and hypertension, and predisposes to athero-sclerotic 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 both 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 2.11 ± 0.20 ng/mg in smokers' hair and 0.22 ± 0.08 ng/mg in non-smokers' hair. Values were 1.93 ± 0.42 ng/mg and 0.51 ± 0.12 ng/mg, respectively. Capillary electrophor- ography measurements indicated a mean nicotine content of 1.24 ± 0.59 mg/mg for smokers and of 0.35 ± 0.11 ng/mg for non-smokers. We found with capilomegas and 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.

Mycophenolic acid: utility of monitoring

R Charfi,1 S Trabelsi3, A Klaouz,* M Lakhal,4 CH Belkahia3 Tunis – Tunisia

Introduction: Mycophenolate mofetil, the active immunosuppressant form of the pro-drug mycophenolic 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 variability 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 the correlation between mycophenolic acid plasma concentration and doses administered.

Methods: It consists on a retrospective study (2001–2005). Plasma levels are measured continuously by high performance chromatography. Therapeutic range is 2.5–4.5 µg/mL.

Results: Four hundred and ninety-four samples were carried from 177 subjects were considered. The plasma level average was 27 years (15–93 years) in 61% men and 59% of 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 corticosteroid-induced 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). In the case of patients who had an insufficient treatment concentration, 19% were in therapeutic interval and 11% were toxic. Statistical analysis showed no correlation between mean mycophenolic acids and dosages 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 accumulation and renal insufficiency. Because of its ototoxicity and nephrotoxicity vancomycin should be monitored when starting therapy. Because of its ototoxicity and nephrotoxicity vancomycin should be monitored when starting therapy.

214 Methotrexate falsely toxic levels T Charfi, S Trabelsi, N Jebabli, A Klouz, I Saloujage, R Daghfous, MH Loueslati, M Lakhal, CH Belkahia – Tunisie

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 optimize the dosing and post-infusion.

Methods: Case-report: We report a case of very high and persistently false 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 leukemia. 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 route prescribed methotrexate (30 mg/week). 10 days after infusion, he came to the out-room department for asthma, vomiting and massive diarrhea. A slow redistribution of methotrexate from extravascular tissues was suspected. Requested methotrexate plasmatic level (carried by the mother) was very high (268 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 alkalinisation 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 didn’t want any medication.

Conclusion: We discovered that the mother added methotrexate to the child blood samples in order to hospitalize her son.


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 2009. The 24 samples were 9.3 ± 3.5 L (6.0–15.5 L) and the median of the last 12-h profile was 1023 ± 1226 days [0–4873]. Children all received an induction treatment and were on ciclosporin, mycophenolate mofetil (MMF) and methylprednisone. MMF was assayed in plasma using an immunnoassay EMTI. 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. The 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 and 12 h post-dose (C0, C1, C2, C3). AUC was calculated as such: AUC = \int C(t) dt from 0 to 24 h.

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 (15.3–174.9). A total of six time combinations were tested. The best LSS was obtained with C1, C2, C3 and AUC equation was defined as such: AUC = 5.759 + 2.584*C1 + 1.034*C2 + 0.616*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. The correlation between MLR and 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 assessed with LSS including C0, C1, C2, C3 and C4 samples only, which are representative for the entire 12 h 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 or 200 mg ritonavir (RTV) (FAPV/RTV).

Methods: This retrospective cohort study was conducted in all the 51 patients from a single center, who had been given OD FAPV/RTV between 1/2005 and January 2007. A sample of at least 2 h after the last drug intake, within first 3 months after FAPV/RTV was started. APV and RTV trough concentrations were assessed using an HPLC method combined with a diode array. Concentrations were 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 mg/mL (IQR 1.13–1.90) and 1.34 mg/mL (IQR 0.91–1.66), in patients given 200 mg (n = 24) and 100 mg (n = 25) RTV, respectively. APV Cmin was <0.75 mg/L 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 < 0.0099). By month 6, no virologic failure was observed among the 13 PI-naïve patients and among the 13 patients who had been switched from OD 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

Introduction: Loratadine (LOR) is a long acting tricyclic antihistamine with selective peripheral histamine H1-receptor antagonist activity. The Cytochromes P450 (CYP) 3A4 and 2D6 have been previously identified as key contributors to loratadine 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: LOR metabolism was studied in pooled human liver microsomes, as well as pooled human liver microsomes, in the presence or absence of chemical inhibitors. The Cytochromes 3A4 and 2D6, that are still controversial in loratadine 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: LOR metabolism was studied in pooled human liver microsomes, as well as pooled human liver microsomes, in the presence or absence of chemical inhibitors. The Cytochromes 3A4 and 2D6, that are still controversial in loratadine metabolism.

Results: Among the 9 isoforms tested only CYP 1A2, 2B6, 2D6, 2C19, 3A5, 3A4 and 3A11 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 (C0 = 12.25 ± 5.5 μM/min/mg) and LOR disappearance (C0 = 115.7 ± 15.45 μM/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 (84.3% and 54.6% respectively). In contrast, quinidine (10 μM) 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

Introduction: Cytomegalovirus (CMV) infection is a frequent complication of solid organ transplantation, usually transmitted by the donor organ, and is responsible for serious morbidity and mortality in transplanted children. CMV infection can be induced by sub-therapeutic GCV concentrations. To minimize resistance, the probability to achieve previously determined target concentrations in PI-naive patients was calculated for each regimen. The probability to achieve previously determined target concentrations in PI-naive patients was calculated for each regimen.

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 sample 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 reinforce 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

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 impact of co-therapy on the lopinavir target plasma concentrations.

Methods: The pharmacokinetics of lopinavir were investigated using a population approach performed with NONMEM on 709 HIV-infected patients (1275 samples). 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-naive and PI-pretreated 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 31.5 L (43.5%) for apparent clearance and distribution volume. 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-naive and PI-pretreated patients was calculated for each regimen. Influence of bodyweight on the probability to achieve these target concentrations was evaluated by logistic regression.

Conclusion: More lopinavir plasma concentrations were reached in patients given a 400 mg BID regimen than in patients given a 200 mg BID regimen when using a 400 mg BID regimen (odds ratios of 0.57 and 0.56 with P < 0.0001).

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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 paediatric patients receiving VRC for ‘proven’, ‘probable’ and ‘possible’ IFI (DIRTC-BAMSI) 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 leukaemia, and one from lymphoma. In three patients Aspergillus infection was documented. No correlation was observed between the genotypes and plasma AUC of VRC. Median VRC dose was 8 mg/kg/day for a median duration of 53 days. Median VRC trough levels was 0.9 mg/L (0.1–1) and median OH-VRC trough levels was 1.2 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 aspergillus 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 sample 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 reinforce the useful of therapeutic drug monitoring in optimizing VRC efficacy.
Conclusion: The 400 mg lopinavir-100 mg ritonavir BID regimen is efficient to reach a mean concentration of 8.3 mg/L for P2T and to PK-AUC.

Results: Before dose adjustment, voriconazole mean plasma concentration was 0.85 mg/L (<0.2–2.7 L/h). 68% of the measurements were lower than 0.8 mg/L. After administration of a same dose of 400 mg (2 x 200), plasma voriconazole concentrations were also from: <0.2–2.7 mg/L. Among the 14 patients, 10 doses were increased as a result of level: one had one delayed elevation of hepatic enzyme which need drug discontinuation; for others increase of dose from 100 to 600 mg, led to plasma concentration of 2.3 mg/L: 200–400 mg: 0.9 mg/L: 100 mg.<br />

Conclusion: Administration of an usual voriconazole dose in cystic fibrosis patients lead to low plasma concentration, no direct link was observed between plasma concentration and necessity 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 model

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Introduction: Stool absorption studies have been used to evaluate oral immunosuppressive 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), ciclosporin A (CsA), tacrolimus (Tacrolimus), rapamycin and mTOR inhibitors (Sirolimus, Siro and Etorimulin, Evoor) using 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 Hanks’ balance salt solution (pH 7.4) 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 Tacrolimus 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. All IS displayed polarized transport. At the lowest concentration tested, the Papp (a–b) was 40–70-fold higher than the Papp (b–a) for calcineurin and mTOR inhibitors, and only 2-fold higher for MPA. IS Papp (a–b) increased whereas Papp (b–a) were decreased with increasing concentrations suggesting saturation of apical efflux transporter(s), except for Tacrolimus 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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Urinary leukotriene E4 excretion: biomarker of active inflammatory bowel disease
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Introduction: Leukotrienes are proinflammatory substances derived from arachidonic acid. Their role in chronic inflammatory disorders collectively referred to inflammatory bowel diseases (IBD). Leukotrienes are prominent in IBD, and are considered to be of diagnostic and therapeutic importance. Consequently, the may be involved in the pathogenesis of IBD.

The aims of this study were to evaluate (1) the urinary excretion of leukotriene E4 (LTE4) in patients with CD, IC and healthy volunteers, and (2) the relation between LTE4 production and the activity/relapse of the disease.

Methods: We prospectively studied 34 patients with CD, 24 patients with IC and 31 sex- and age-matched control subjects. Activity of the disease was determined on inclusion by Cohn's disease activity index for CD and clinical index activity for UC.

The urinary excretion of LTE4 was measured by liquid chromatography tandem mass spectrometry. LTE4 data are expressed as pg/mg protein and presented as median (10th–90th percentiles).

Results: The urinary excretion of LTE4 was increased (P = 0.0016) in patients with CD: 54 (25–125) and UR: 64 (26–186) compared to healthy subjects: 33 (21–82). LTE4 levels were significantly (P = 0.01) higher in patients with active disease than in patients with relapse for whom the levels of LTE4 were similar to the level of healthy subjects.

Conclusion: 5-lipoxygenase pathway activation could contribute to the inflammation associated with IBD. The quantification of urine LTE4 could be an interesting non invasive biomarker for the assessment of the biological activity of CD and IC.

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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 immunosuppressant 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 Aponyme, Operma). 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 valid. The aim was to validate a new method to adapted 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 limiting errors. Thus there is a definite 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 fractional 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 boundaries. I.e. (AUC0-12 h boundaries, i.e. 95% of cases).

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. For the last 3 months, all post-transplant patients, more than 90% of patients were under the AUC0-12 h target range. In patients with a AUC0-12 h dose was more than 1 to 10, with 47 to 54% patients outside the acceptable therapeutic range before dose adjustment. A majority of patients were underdosed during the first 3 post-transplant periods, while the distribution between underdosed and overdosed patients was found later on. When patients were seen again after a first dose adjustment and the dose was given (still in the previously proposed dose range, i.e. 2/3 of cases), 90–95% of 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 1/3 of cases. Collaborative pharmacokinetic studies are ongoing 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), often with fatal anasthesiologic consequences. Ondansetron, a serotonin type 3 (S3) receptor that can cause nausea or vomiting or both, could cause dopamine type 2, serotonin type 3, histamine type 1, and mucusin cholinergic type 1 receptors. Two effective agents are droperidol, a dopamine D2 receptor antagonist, and haloperidol, a dopamine 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 two antimotion drugs might be the only solution 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 establish 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, 10) using an acetonitrile/0.1 M ammonium phosphate buffer 0.1% in water (40/60 v/v) at 1.0 ml/min 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, or placebo. Blood samples were collected at the end of perfusion and after 1, 2, 3, 4, 6, 8, 10, 12, 24, 48, 72, 96, and 120 h.

Results: Retention times were 2.63 min for ondansetron, 2.50 for tropisetron, 5.17 for droperidol and 4.77 for haloperidol. Calibration curves were linear for both compounds in the 0.50–50 ng/mL range. The limits of detection and of 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 0.4% and 1.6% for ondansetron and 1.2% and 1.0% for haloperidol. Inter-assay accuracies were in the 97.6–103.9% range either at 3. 10 or 100 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.
231 Drug perception by children: a survey of 138 children at school

Introduction: Drug consumption is considered as heavy drug consumption, 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 they 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 children when taking a drug.

Conclusion: Children hardly know drugs. Discussing drugs at school as soon as possible could improve knowledge and promote rational drug use.

232 Pharmacoepidemiology of over-the-counter drug abuse and dependence: a pilot study

Introduction: Over-the-counter (OTC) medicines are used to treat minor pathologies (pain, infection, etc.) by the patients themselves without prescription and some of them contain psycho-active substances. The aim of this pilot study was to develop a pharmacoepidemiological method to investigate abuse and dependence of OTC medicines. This method was 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, abuse and dependence according to the criteria of the 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. Tobacco use will be considered as a confounding factor. We expect a difference in the number of cases of abuse and/or dependence between the control group and the other ones. Pharmaceuticals 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 category, previous psychiatric drug 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.

233 High dosage buprenorphine consumption after ten years of marketing.

Introduction: In France, two parenteral programmes for opiate users are available: the Methadone Programme (with high dosage buprenorphine) and the Opiate Treatment and Social Rehabilitation Programme (OTSRP). This study assessed since ten years since 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 patients using BHD.

Methods: The Opiate Treatment Programme (OSSP) is an ongoing observational study of illegal drugs and misuse of psychotropic medications, a multicentric survey, annually surveys drug dependent subjects in treatment (mainly methadone and heroin addicts) or drug centres caregivers (mainly heroin addicts). BHD misuse was 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 = 1111), 94% (n = 1231) subjects were identified as ‘within protocol consumption’, consuming the drug in a strict care protocol, with a correct follow-up and 6% (n = 78) ‘outside protocol’ (consuming the drug without any supervision). Many differences were noted between the two groups. For the subjects (within protocol consumption, n = 133) we daily daily dose in 2005 was 15 mg (range 9.4% in MP and 9.4% in PACA), 70% only with OTHAs (67.3% in MP and 75.8% in PACA) and 13% with the injection of insulin. By contrast, OTHAs were more frequently used in PACA (88.5%) than in MP (81.3%; P = 0.001). Among OTHAs, the sulfonylureas and metformin were the most commonly used (60.3% and 45.8% respectively), followed by the a-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 OTHAs in MP and 86.8% in PACA. Concerning the consumption of cardiovascular drugs, over the age of 80, antidiabetic drugs were consumed by half of the diabetics with 72% receiving statins and 28% librates. ACE inhibitors were used by one third of the patients (31.5% 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 treated with OTHAs 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.
236 Data collection agreement in pharmacoepidemiology: comparison of patient, physician and database sources

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 records, 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) data.

Methods: Between the 1 of August 2002 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 also extracted from the French national health insurers database. Were compared: medical histories and NSAID indication as reported by the patients and the physicians; and type of prescription initiation or renewal of treatments as indicated in the three sources. Concordance was poor for gastrointestinal history (K = 0.63). Concordance was medium for report of hypercholesterolemia (K = 0.53, patient vs. Physician), for type of prescription (K = 0.53 patient vs. Physicians), and K = 0.57 for GP vs. Database. Between patient and physician, concordance was poor for gastrointestinal history (K = 0.55) and for osteoarthritic indicators (K = 0.52). Concordance between patient report and the database was good for the patients' age but 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 (gastrointestinal disorders). Discordances between patient and database for gastrointestinal protection could be due to self-medication by the patient.

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

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 are used to use medicinal plants for the treatment of their health problem. 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 usage of medicinal plants for the treatment of diabetes in Senegal in order to make recommendations for their best use in their number.

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 medicinal plant(s), the reasons of using it, effects of the plant on 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 230 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 (63.90%), Solanum hircirra (41.20%), Allum satium (6.8%), Terminalia avicennioides (5.5%) and Garcinia cola (5%). Patients declared several reasons for using medicinal plants (traditional treatment: 40%, efficacy: 12%, low cost: 20%). The principal suppliers of plants were in the market (66.8%), traditional therapists (5%) and structures of traditional medicine (1%). 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 people have access to medicinal drugs. In addition, in Africa, traditional believes are used to use medicinal plants 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 44.9% on adverse effects. Twenty percent (20%) had advices which could be caused by medicinal plants. These adverse effects were in general digestive 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.

238 Non rational use of high dose buprenorphine: Comparison of use’s pattern between 2000 and 2005
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Introduction: Results have reported high buprenorphine (HBO) misuse. ‘Non rational’ users have been associated with an increased misuse of HBO or benzodiazepines. In France, ease of access to HBO has contributed to diversion to the illegal street market, drug shops and doctor shopping. Intensive use of buprenorphine practices and Health System regulatory interventions were started 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 HBO users identified from the French Health System reimbursement database in Haute-Garonne between January 2003 and October 2005. All drug deliveries were followed for 6 months. Patients were considered as rational, non rational or occasional users according to the French Health System reimbursement database for gastroprotection was borderline (K = 0.53, patient vs. Physician), for type of prescription (K = 0.53 patient vs. Physicians), and K = 0.57 for GP vs. Database. Between patient and physician, concordance was poor for gastrointestinal history (K = 0.55) and for osteoarthritic indicators (K = 0.52). Concordance between patient report and the database was good for the patients' age but 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 (gastrointestinal disorders). Discordances between patient and database for gastrointestinal protection could be due to self-medication by the patient.

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

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 hospital 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 diabetes mellitus (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 other cases, spironolactone was used after. In the group of non rational users, HDB daily delivered quantities have increased during the follow up. We compared these patients to those included in a previous cohort with the same design and with the same data collection. We 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 and with the same data collection. We 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 and with the same data collection.

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 cases due to a lack of surveillance of ambulatory patients treated with drugs known to raise potassium plasma level.

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

Introduction: Prevention of drug-related adverse drug reactions (ADR) 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 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 care units. If adverse drug reaction was severe, a physician was asked to pay 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 136 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. Data were available in 90% of the cases. Spontaneous notification rate is in 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. Despite data in medical records, stratification of morbidity 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 unit, the transmission of adverse drug reaction appears to be a loss of data. When mentioned at arrival, 81.5% of ADR are mentioned in discharge summaries. 31.5% explicitly written.

Conclusion: Computed chart existence since several years and participation to such evaluations show engagement of emergency units in quality enhancement. Flaws possibilities exist: delay of information transmission because of delay of transmission of information to patients and physicians, because of inhomogeneous 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.

241 Zolpidem abuse: regional health insurance database analysis
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Introduction: Zolpidem, available in France since 1997, is an hypnotic medicine which therapeutic treatment is limited now to one 10 mg pill a day. The aim of this study was to describe antiparkinsonian drug use in Parkinson’s disease regions.
Methods: Analysis of data from ‘Bouches-du-Rhône’ department health insurance database from January, 2000 to September, 2004. 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 INSEE software with an anonymised analysis.
Results: One hundred and twenty-five patients ingest an average value of three pills a day and 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 1 to 79.

The average number of medical doctors consulted for prescription is 8.3 (from 1 to 75 for men and from 1 to 73 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 125 patients were seen by the medical practitioners. 70 of them declared 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 16% 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 misuse and/or dependence zolpidem. The consultation with the insurance medical practitioner seems to have a positive impact on abuses.

242 Treatment in Parkinson’s disease – Pharmacoepidemiological research into the use of parkinsonian drugs
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Introduction: This study was the part of a prospective study of 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 (Côtes d’Azur, CA)].
Methods: We selected all patients receiving antiparkinsonian drugs or anticholinergic drugs during 1st October to 31 December 2004. Antiparkinsonian drugs were levodopa, dopamine agonists (bromocriptine, pergolide, ropinirole, pramipexole, lisuride), anticholinergics (Biperidene, trihexyphenidyl, tolcapone), selegeline and entacapone. Patients younger than 35 years old and receiving only anticholinergic and neuromuscular drugs were excluded. Drug reimbursements were collected for the year 2003.
Results: The study identified 4162 PD patients in MP and 7304 in PACA. About half were men, their mean age was 62.8 years with a range from 5 to 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 database of the health insurance system. Levodopa was most frequently used in MP (85% 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). Selegeline 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% in dual therapy and 10% in triple therapy. In PACA, Levodopa was more frequently used in older patients (65% in patients <70 years to 90% in patients >90 years) whereas dopamine agonists were frequently used in younger 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.
Results: A total of 811,900 ‘social policy-holders’ was recorded in the regional database from which 33,313 ‘social policy-holders’ have received at least one prescription for hypnotic or sedative drugs of phytotherapy in the previous 6 months before the end of their refund. The evaluation 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 total 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 this follow-up for sales of the drugs of phytotherapy 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 mortality; (iii) non coronary mortality. We compared the prediction to the observed incidence of events in the French population.

Conclusion: 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.

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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 considered (i) demographic statistics. Endpoints were (i) total cardiovascular mortality; (ii) coronary mortality; (iii) non coronary mortality. By using probabilistic algorithms we re-created virtual but realistic individuals linking the risk factors to the incidence 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) the demographic statistics; (ii) a specific cohort; (iii) linking the risk factors to the incidence of cardiovascular events.

Conclusion: These results seem to contradict the assumption that consumption of drugs of phytotherapy would have referred on other refundable specialties.

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Prevalent 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 (ADR) in real life from a three sources capture-recapture method and estimate the 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 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 castolog 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 (ICD). We excluded cases suggesting non-adverse drug reactions. The number of cases 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. A

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 independent model (without interaction between the three sources), for which confidence interval was the narrowest, Akaike Information Criterion was the lowest (−3,37). In the sensitivity analysis, the lowest too was −9,02. The number of lacking cases was 913 (397–1429), very near of the number obtained with the weighted Bayesian Information Criterion (901). The number of total cases was 128 (702–1734). During this period, 9489 inpatients were registered. So prevalence of adverse drug reactions during February is 12.8% (7.4–18.4). Exhaustivity of discharge summaries was 20.1% (14.1–14.9), of the programme de médicalisation des systèmes d’information 4.7% (3.8–5.1) 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 pharmacopoeiologic studies, it has not been conducted. Spontaneous reporting rate is low compared to previous studies. Discharge summaries appear to be a promising tool for adverse drug reaction detection and survey.

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Assessing general practitioner’s prescribing behaviour in elderly patients with uncontrolled renal failure

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Introduction: In elderly patients serum creatinine level may be normal despite kidney function is reduced (renal filtration failure, RFF). For example 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. Our study was considered this issue in general practice. The objective of this study was to assess general practitioner’s (GP) prescribing behaviour in elderly patients with uncontrolled 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 selected to achieve a questionnaire including clinical cases simulating drug prescription in elderly patients with uncontrolled 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 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 if the serum creatinine level is normal (while it was decreased) and only 14% of them really calculate clearance with the Cockcroft 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 levoloxacine (48%). For the prescription of a 70-year-old patient whose clearance was estimated by the Cockcroft and Gault equation. 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 means 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 medication classes.

Methods: We extracted all drug deliveries reimbursed by the General Health Fund during year 2005 in PACA-Drôme region (population 2.9 millions) for oral route formulations of five classes of psychotropic medications (benzodiazepines, anti-depressants, opiate maintenance therapy, stimulants and neuroleptics). We used two indicators to evaluate the abuse potential of each drug 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: The GPs displayed substantial doctor shopping for all classes (mean doctor shopping ratio 12.1% for benzodiazepines, 12.8% for neuroleptics, 13.6% for anti-depressants, 21.7% for stimulants and 25.9% for opiates). The compounds with the most important doctor shopping ratios were: buprenorphine for opiate maintenance therapy (14.1%, 462 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 the maximum doctor shopping ratio for methylphenidate (2.4%). Abuse potential of neuroleptics is also high.

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Reference 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 best way to improve control is to optimize the ‘controller’ medication. In this context, the controller medication may be less effective for patients with Infants with asthma who do not use long-acting inhaled β2-agonist (LABA) in a single inhaler (ASSO), (iii) ASSO in association with LABA in separate inhalers (DISSO), (iv) Antileukotrienes (ALT).

Patients included in the DISO group had renewal prescriptions of ICS and LABA within an

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interval of 30 days. For each patient, we collected socio-demographic 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 non-persistence. Treatment persistence was defined as the cumulative 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. The index date was the end of the calendar date of prescription and the start date of the following one. For groups treated with ICS (alone, combined or dissociated), discontinuation was considered when 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 = 15), 56% were women. 43% were new users. CMA was below 80% for 85%, 79%, 62% and 61% of new users of ICS, ASSO, DSSO and ALT respectively, and for 70%, 46%, 24.5%, 26% of previous users. Persistence rates were 17 months for 20%, 31%, 78%, 38% for new users of ICS, ASSO, DSSO, 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.

251 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 data from the French National Drugs and Misuse of Psychotropic Medications performed in October 2005.

Methods: OPPIDUM is a national, and multicentric survey describing consumption and abuse of psychotropic drugs (Centers of Evaluation and Information on Pharmacodependence) organize the data processing and their collection from drug addict patients met in health care centers.

Results: Consumption characteristics of BZD have been analysed and particularly misuse indicators (search of positive effects, concomitant intake of alcohol, illegal acquisition) were high. The more used were bromazepam (127 times reported), clonazepam (115), diazepam (111), oxazepam (108), diazepam (98), alprazolam (98), zolpidem (76) and flunitrazepam (59). Bromazepam (127 times reported), clonazepam (115), diazepam (111), oxazepam (108), diazepam (98), alprazolam (98), zolpidem (76) and flunitrazepam (59).

Conclusion: Consumption characteristics of flunitrazepam and clonazepam are remarkably different of other BZD, underlying a higher misuse for this two drugs. If to recommended dose, 82% of abuse/dependence, 89% of positive effects, 56% of (111), oxazepam (108), diazepam (98), alprazolam (98), zolpidem (76) and flunitrazepam (59).

252 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: 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. Data were also used from these studies in order to define a 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 observed. The aim of this study was to investigate the 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 menopause were excluded as confounding factors. Information on acquired/inherited risk factors were collected in a face-to-face questionnaire and a blood sample was drawn to evaluate biological status. 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 (OR, 2.1; CI, 1.4-4.1). Progestin-only contraceptive use accounted for, respectively, 22.2% and 77.8% of DCs at risk of TD. Prematurity of orders for nonpsychotropic drugs potentiating the risk of TD was similar in the GP group treated with QT/APS and in the GP group receiving other antipsychotics (i.e., non-TD-prolonging antipsychotics), respectively, 53.8% and 38% (P = 0.2).

Conclusion: First, a large proportion of patients exposed to antipsychotics in the geriatric population (P = 0.01) of a 415-bed teaching psychiatric hospital.

253 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 may require multiple drug therapy. The aim of the present study was to evaluate the prevalence of these DCs at risk of TD in the geriatric inpatients (n = 265 years) of a 415-bed teaching psychiatric hospital.

Methods: The study consisted of a 1-day cross-sectional evaluation of all the ongoing drug regimens recorded by the patients during the 60 days before the second day study day. Patients were then stratified into 3 groups: patients weighing < 50 kg, 50–70 kg and > 70 kg. Drug combinations (DCs) at risk of QT prolongation and torsades de pointes may especially occur in case of polyphearmy 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 TD in the geriatric inpatients (n = 265 years) of a 415-bed teaching psychiatric hospital.

Results: In total, 152 GP were included: 102 received antipsychotic therapy of whom 52 were treated with QT/APS (prevalence 5%, 95% CI 4.1–6.0). In this QT/APS-receiving GP group, 16 cases of QT/APS polypharmacy or QT/APS-containing DCs at risk of TD were recorded, giving a global prevalence rate of 69.2% (95% CI 56.6–81.7). There were eight cases of C/ICDCs due to QT/APS polypharmacy or combinations of QT/APS with amiodarone. QT/APS polypharmacy and combinations of QT/APS with bradycardia-inducing nonpsychotropic drugs accounted for, respectively, 22.2% and 77.8% of DCs at risk of TD. Prematurity of orders for nonpsychotropic drugs potentiating the risk of TD was similar in the GP group treated with QT/APS and in the GP group receiving other antipsychotics (i.e., non-TD-prolonging antipsychotics), respectively, 53.8% and 38% (P = 0.2).

Conclusion: First, a large proportion of patients exposed to antipsychotics in the geriatric population of a 415-bed teaching psychiatric hospital.

254 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 31st of May 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 2003 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 demography, diagnosis, concomitant drugs, and the presence of nonpsychotropic 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 antidiabetes concomitantly with a statin; patients with cardiovascular diseases (PDC) and patients without diabetes or cardiovascular diseases. The latter were considered as patients treated for primary prevention.

Results: 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 of statin treatment.

Results: We identified 16, 307 new users of statins (M/F sex ratio: 0.92) representing 15% of the whole population of treated patients in 2004. The median age was 58 years (± 53.5) for men and 63 years (± 53.8) for women (P < 0.01). Pravastatin was the most prescribed drug and general practitioners the most numerous prescribers (67.1%). CP represented 46% of the study population, 42% were classified as diabetic patients (DP), 37% were patients with diabetes and cardiovascular diseases (PDC) and patients without diabetes or cardiovascular diseases. The latter were considered as patients treated for primary prevention.

Conclusion: Persistence decreases early in the statin treatment and is higher for patients with few other cardiovascular risk factors.
Methods: Severely hospitalized patients treated with statins were interviewed. Several aspects were collected: autodrugs, automedication, medical history, the patient, treatment patterns, knowledge and perception of the treatment by the patient, the physician/patient relationship and adherence to treatment regimen.

Results: A total of 30 685 patients were interviewed, 14% of those were under 30 years, 23% were women and 66% for men (extreme: 45-93). Prevalence of cardiovascular risk factors was high (systemic hypertension: 73.4%, diabetes mellitus: 27%, cardiovascular disease: 30.5%). Among patients treated with statins, 77% were using them for more than 1 year, 30% for 1-2 years, 19% for 2-3 years, 8% for 3-4 years, 6% for 4-5 years, 5% for 5-6 years and 9% for more than 6 years. Only 50% of patients had knowledge of their treatment by statin. Among those having knowledge of their treatment, 20% knew that this treatment was for cholesterol, 24% in the prevention of acute coronary events and 45.4% that the objective of monitoring cholesterol levels was to limit the risk of myocardial infarction or stroke and 45.4%, 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 they didn’t need it when 71.3% 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 assessed the adherence of interviewees by interpreting 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.

Methodology of a prescription forgery survey

Introduction: The French network of Centres d’Évaluation et d’Information sur la Pharmacodependance (CEIP) performs since 2001 a prescription forgery survey with community pharmacies networks. The system called OASIP (Ordonnances Suspectes Indicateur d’Abus Possible) provides information about potential abuse liability of marketed drugs in France. The collection of these data was part of the French communal pharmacies participation. We began in January 2003 a European collaborative project funded by the 2003–2008 Public Health Program of the European Commission, to extend the OASIP system to invasive European countries. The aim of this project is to compare temporal and spatial 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 specialties and dosages available, as well as the scheduling and rules of prescription in each country.

Methods: The participants develop 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 in 2 months/year.

Results: The first collection data was done in May 2006. Around thirty pharmacies participated in Belgium (covering 150 000 inhabitants), Italy (110 000), the Netherlands (300 000 inhabitants) 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 fluconazole (Belgium), alprazolam, methylphenidate and clonazepam (Spain). The drugs most often reported were narcotics (or drugs with similar scheduling) in the Belgium collection, three of these drugs being not marketed in France.

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 a non-prescription country), it will be interesting to compare results of prescription forgeries survey and level of use.

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

Methods: Cross-sectional observational study carried out according to the methodology ORP® (MedISCAN): 1051 general practitioners randomly selected collected using 6688 all subjects presenting with hypertension for 2 months in the 176 uncontrolled hypertensive patients. During the collection of the data, missing or incomplete informations were the subject of requests. Totally 5.4% of the patients received diagnoses 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 3 years. 55% of the patients were overweight or obese (BMI ≥ 25); 44% had only one another CVRF. 56% had two or
more. The CVRF were hypertension (51%) (86 of which were under treatment), sedentary (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. Anti-hypertensive 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%) and others.

Conclusion: This study highlights the importance of the insufficient control of the blood pressure and the undermanagement of associated CVRF in hypertensive treated patients.

260 Drugs in general practice: patients' expectations, doctors' perceptions and related-behaviour – a questionnaire survey A Sommer, C Maillet, P Lambrichts, M Lapeyre-Mestre, IL Montastruc, a Toulouse – France: b Verdalle – France

Introduction: In France, a large majority of medical consultations ends with a prescription of medication. Previous studies have revealed that some doctors' practices 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 consultation with their regular 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 (1980/2272) of the patients reported at least one switch. Only 9% (202/2272) did not expect any switch for each consultation. When doctors decided not to prescribe any drug, 83% (1532/1836) of patients agreed with this decision. If no drug was prescribed, several proposals of generic further inferior in price (47%), further equivalent but cheaper 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. Clinically statistically significant differences were found between sex groups.

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 relationship to improve health care and give other answers that the pharmaceutical one to multiple patients' expectations.

261 Analysis of pharmaceutical dispensation in chronically treated patients with drugs registered in the generic drug list P Laine-Cessac, a C Monicard, JF Benoist, L Lagarce, c Bruhat, b Biquet a Angers, France; b Bordeaux, France

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 25%. For some drugs, generic substitution is a mean of increasing the generic substitution in order to increase generic competition, fix price for generics and so on. The aim of this study was to compare the number of switches from traditional non-steroidal anti-inflammatory drugs (tNSAIDs) to Coxibs in the CADEUS study.

Methods: The study was observational and used computerized records of the general health insurance system (CPAM) of Angers. Four AP were selected for their participation in the study period.

Results: Carbamazepine was used in 189 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 1.0. Cimetidine 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 1.0. The mean number of switches was 1.1 (0–11) with a median at 0.0. 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 1.0. The mean number of switches was 1.0 (0–11) with a median at 0.0. 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 (1–12) with a median at 2. The mean number of switches was 1.0 (0–12) with a median at 0.0. 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 drug. The number of prescriptions 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 number of DDP and switches are less than two and finally the risk of misuses is probably less than was feared.

262 Switching from traditional non-steroidal anti-inflammatory drugs to Coxibs in the CADEUS study C’Droz-Ferrouste, H Nguyen Hoang, F Departot, R Lassalle, J Jove, b P Blin, a N Montastruc, a A Fourier-Régat, a Toulouse – France

Introduction: Using data from the CADEUS study, we studied the frequency of switches from traditional non-steroidal anti-inflammatory drugs (NSAIDs) 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 Paris General Health Insurance System for salaried persons (CNAM-TS) who had a claim for NSAIDs (date of claim = index date) were included in CADEUS. This study was performed on a sub-population of the cohort, composed of subjects within the Cox-2 inhibitor claims. The 6 months before 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 switch, socio-demographic, medical and drug delivery data were considered. Characterization of switches was performed using logistic regression analysis.

Results: Of 11 553 NSAIDs 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 = 1.1–1.95; 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 NSAID to Cox-2 inhibitor concerned <10% of subjects. Switching is most important in subjects known to be at risk of gastrointestinal adverse events.

263 Misuse, abuse and dependence of psychotropic drug in France – From OPPIDUM program of CEIP Network P Laine-Cessac, a L Lagarce, M Roudier, b S Pomies, a JL Montastruc, a L Lagarce, c Bruhat, a B Biquet a Angers, France; b Bordeaux, France

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 psychoactive medications) is a multicentric survey, annually surveys drugs 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 drugs 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 abused: 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 (napicoline and aldipadin) (24–30%) 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), ilotiapine (14% description of abuse or dependence) and tiapine (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 cannot be generalized to the whole population. Nevertheless these data are precisely interesting due to the characteristics of this population with history of abuse.

264 Patients at risk of post-operative nausea and vomiting (PONV): evaluation of a predictive score M Mottila, a B Basset, K Delanoue, b B Bellon, s P Pomes, d Chenevier a Toulouse – France

Introduction: Post-operative 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. 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-operative period, PONV were collected during 12 months. Data concerned AS, surgical and anaesthetic risk factors and PONV morbidity. All surgical wards were concerned including paediatrics, obstetrical and ambulatory surgery. Patients excluded were those under 18. Data were collected by questionnaire on socio-demographic variables and drug use during the preceding week. Different classes of psychotropic drugs have been studied: benzodiazepines (BZD), other anxiolytics or hypnotics, antidepressants and neuroleptics.

Results: Among patients included, 10% were excluded leaving 133 questionnaires to analyse. Incidence of PONV was 57% (7/133). The number of patients who benefited of a prophylaxis, a histamine type 1 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 (98%). Predictive value of the 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.

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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.

265 Clinical description and therapeutic management of tuberculosis in patients of HIV coinfection with the human immunodeficiency virus (HIV)

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Introduction: Management of tuberculosis in 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/mm³, mean plasma viral load of 6.2 log10 copies/mL), 77 of them (69%) were from Sub-Saharan 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) and rifampicin (n = 1) and to ethambutol (n = 1). Among the 53 patients in whom tuberculosis revealed HIV infection, HAART was introduced in 44 cases 2.6 months upon antibiotic treatment start. Drug-related adverse events occurred 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.

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.

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

A Perrin-Terrin1, A Pathak2, C Arbus3, JL Montastruc3, M Lapeyre-Mestre4 *Toulouse – France

Introduction: Recent case series suggest the role of synthetic opioid methadone in QTc prolongation and torsades de pointes 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 planned, 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-variables such as age, sex, methadone dose. The main outcome was QT interval measured on ECG, 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 treated 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 ± 13 ms (range 15–145). A prolonged QTc and QTc dispersion were observed in 7% and 12% of the patients. Thirty-nine controls (9%) were exposed to psychoactive drugs known as prolonging the QT interval. One patient was treated by buprenorphine. We will investigate of 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. Taking into account the study design, a prolongation by buprenorphine or without opiate maintenance treatment could confirm our results and conclude to a causative relationship between methadone and QT.

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

V Brenet-Dufour1, V Gras-Champel2, H Masson1, M Andjekaj2 *Amiens – France

Introduction: In 2000, 73.6% of the women from 18 to 44 years state to use a contraceptive method. The most largely used (45%) hormonal contraception has different ways of administration (pill, intra-uterine device, ring,
and palp). Many drugs interact with the hormonal contraceptives. Some of the interactions are well known, 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 hormonal contraception, reported to the Regional Pharmacovigilance Center (CRPV) of Amiens.

**Different drug interactions, proved or potential, being the cause of a lack of effectiveness observed.**

**Results:** First case: interaction between ethinyloestradiol/levonorgestrel and carbamazepin, pregnancy discovered 2 weeks of amenorrhea. Second case: interaction between etonogestrel (implant) and rifampicin, pregnancy discovered at 25 weeks of amenorrhea. Third case: interaction between levonorgestrel and bosantan, pregnancy discovered at 13 weeks of amenorrhea.

**Review of literature:** The mean listed interactions are linked to enzymatic induction but other mechanisms may 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 consistent 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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**270**

The drug compliance – a ‘random’ variable whose taking into account is essential

**Introduction:** Drug compliance (oral anti-hypertensive therapy) is an important factor in the cardiovascular prevention. The non-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 constant 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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**271**

Allopurinol-induced severe systemic hypersensitivity reaction (DRESS syndrome)

**Methods:** M Gumyaa, P Netter, P Gillet

**Introduction:** The drug hypersensitivity syndrome, or DRESS syndrome (drug rash with eosinophilia and systemic symptoms), is an uncommon but potentially life-threatening drug reaction. DRESS is a severe toxidermic reaction accompanied by lethal visceral involvement in 6 to 10% cases. 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 anticogulation are assessed.

**Results:** With these clinical cases, was illustrating the fact that the ‘actual’ compliance is more complicated and more frequent than expected. The compliance or even the low compliance can be a cause of severe adverse drug reaction.

**Conclusion:** Our solutions seems to be a repeated training and a constant 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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**272**

Adverse drug reactions of methylphenidate in French Pharmacovigilance database

E Herlem*, V Marchaisseau*, ML Germain*, T Tremou *Reims – France

**Introduction:** Methylphenidate is a pipeline 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 diagnosis of ADHD and the treatment of ADHD are controversial. Neurologists, psychiatrists and paediatrician, only, are allowed to prescribe methylphenidate which is covered by ‘narcotics’ instructions.

**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 (45.8%) 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’ were reported. Two growth suppressions are observed. Only one alopiecia and one thrombocytopenia are notified. In adult, is addiction is reported in three cases, off label use in eight cases. Convulsions: The profile of seizures is variable and seizure types depend on the type of methylphenidate. The limitation of spontaneous reporting is essential in the assessment of psychiatric adverse events due to the high degree of co morbidities (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 investigation are mentioned in the ‘warnings and precautions for use’ section of the Summary of Product Characteristics. Long-term studies, over 3 years, are needed.

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**273**

A case of serotonin syndrome caused by venlafaxine and lithium (Serotonin Syndrome). P Netter*, S Ponvez**, P Gillet

**Introduction:** The Serotonin syndrome is a toxic hyperserotonergic 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 that has suffered from a serotonin syndrome secondary to venlafaxine and lithium 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 possibility that this might have occurred due to a lithium treatment, no symptoms were observed. The syndrome was resolved completely after the interruption of venlafaxine administration. Lithium therapy was carried on without notable problem. An observation of a patient that has suffered from a serotonin syndrome secondary to venlafaxine and lithium valxafaxine in order to discuss the underlying mechanisms.

**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-serotonergic 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 have lethal outcome.

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**274**

Loss of sight: a side-effect of bortezomib?

C Soignon*, MC Perrin*, D Bertram* "Lyon – France; Bourg-en-Bresse – France

**Introduction:** The proteasome pathway plays an essential role in the degradation of the majority of cytoplasmic, nuclear and membrane proteins. Bortezomib is a novel proteasome inhibitor that specifically inhibits the proteasome function and it is now becoming commonly used in haematological malignancies.

**Methods:** 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 combed by a rapid death. On 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/m2 at days 1+4+7+11, on a 21-day cycle basis. At the beginning of the...
March 2006, because of a severe thrombocytopenia, it was decided to stop definitive therapy in March 2007, at the patient's request. The patient was monitored every 2 months. At the end of March, the patient developed a peripheral polynéuropathy together with a sudden central scotoma of the left eye. During the following week, he developed a sudden loss of visual field of the left eye. After 1 month, visual field of the left eye was partially recovered. Examination showed a partial blindness (0/10) of the left eye and partial blindness of the right eye (0.8/10) caused by a macular scotoma. 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 spontaneously and without treatment. On November 11, 2006, it was stated that the patient had recovered with sequelae as his retina was permanently damaged.

Conclusion: 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 maintained the bortezomib therapy without any new exploration. For his previous rituximab treatments. Furthermore, rituximab is a potential treatment for patients suffering from optic neuritis. 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.

275 Tumour necrosis factor-alpha antagonist, etanercept and demyelinating disease: analyse of French database and review of the literature
G Veyrac*, G Cognet*, P Jolliet* "Nantes – France"

Introduction: The tumour necrosis factors (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 demyelinating 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 as ‘suspect’ according to the OMS score. These notifications concerned 16 women and five men; the middle-age is 51.7 years (13 - 77). 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 unknown 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 a new observation was observed in seven patients.

Conclusion: Physicians must screen candidates for TNF-α inhibition therapy carefully to exclude those with symptoms, signs or family history of demyelinating diseases. Anyway, prescribers must watch over their patients and stop the treatment in the case of a demyelinating occurrence. In case of 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.

276 Hypersensitivity to bortezomib: a case report
A Chevalier*, S Dulaç*, E Collet*, P Bamberger* "Lyon – France; 2Bijn – France"

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 ever 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/m² bortezomib twice weekly for 2 weeks. After 2 weeks, the third injection of bortezomib, the patient developed an isolated cadavus 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 occurred in 2 weeks. A cutaneous biopsy of the lesion evidenced a diffuse mononuclear infiltrate including histiocytes and fewer T-cells and showed typical histological and immunological arguments for a skin involvement of his lymphoma.

Conclusion: Physical and histological arguments for TNF-α inhibitor therapy carefully to exclude those with symptoms, signs or family history of demyelinating diseases. Anyway, prescribers must watch over their patients and stop the treatment in the case of a demyelinating occurrence. In case of 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.

277 Intravenous immunoglobulins-induced eczematous eruption: a long-term-follow up study

Introduction: High-dose intravenous immunoglobulins have emerged as an important therapy for various diseases. Visceral eczematous eruption has recently been described in three cases as a rare adverse event of this treatment. However, it is well known that patients’ characteristics, administration regimens and long-term outcomes.

Methods: We retrospectively examined from medical charts of 20 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 3 days for the remaining two patients. Eczematous 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). Eczematous eruption was mostly localized on hands (85%) and was usually pruriginous in seven patients. All patients improved, either spontaneously or with steroid treatment, with a mean delay of 21 days (range: 10–10). Immunoglobulins were re-administered in three patients, in all cases in which the skin eruption occurred. The patients were re-challenged, with a mean delay of 21 days (range: 10–10). Immunoglobulins were re-administered, with a more widespread eruption in all. However, eczematous eruption was attenuated from the third administration. Eczematous 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 pruritus.

Conclusion: Eczematous eruption caused by infusion of immunoglobulins 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 percent were treated for neurological disease. As in our series, it is usually localized on hands (85%) and soles (15%) and was usually pruriginous (81%). Patients were commonly treated with topical (45%) or systemic (14%) cortico-steroids, although others improved without treatment. Eczematous eruption was mild and did not require hospitalization.

278 Severe cardiac events associated with the discontinuation of nadolol treatment in three patients

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 crisis. We report 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.

Methods: Case report.

Results: Case 1: A 11-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 mechanical assistance 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 by 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 therefore attempted but were unsuccessful. 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 atenolol (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 particular profile of action. This case report is led to question the particular profile of action of this beta-blocking drug in ventricular arrhythmias in the setting of chanelopathies 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-blocker may be deleterious in selected patients.

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Ischiococclidean and neuroleptics – a retrospective study in a psychiatric hospital and a new physiopathologic hypothesis


Introduction: Ischiococclidean is a very serious side-effect of neuroleptics, particularly in young patients. The aim of this study was to have a better knowledge of this side-effect in order to improve management and to evaluate ischiococclidean frequency.

Methods: A retrospective analysis was done in patients with ischiococclerosis who 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 ischiococclerosis. We attributed a gravity level to ischiococclerosis 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 chiefly (7), abdominal pain (6), vomiting (6) and occlusive syndrome (2). Diagnosis was based on clinical symptoms (11), colonoscopy (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. A close association with neuroleptics had been observed at least on one occasion. In spite of different ischiococclidean dosages. Furthermore, we estimated the ischiococclidean 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. Dopaamine 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-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.

Materials and Methods: In 2005, the French Association of Regional Centres of Pharmacovigilance (CPRV) has decided to maintain the sirolimus 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 grained 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 use of sirolimus in liver transplantation 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 indeed eventually be reversed by sirolimus withdrawal or sirolimus. 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 opioid 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 hospitalisation from 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: In France, the National 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 versus 706 DDD/1000 D in 2000. The use of dextropropoxyphene in association with paracetamol was the second most used analgesic after paracetamol. Although decreasing since 2002, its consumption was 139 DDD/1000 D versus 186 DDD/1000 D in 2000. In 2006, after dextropropoxyphene withdrawal, the use of tramadol increased in comparison with 2004. This increase involved tramadol associated with paracetamol. The overall consumption of step 2 analgesics decreased by 22%
Thirty-six patients were treated by danaparoid. 45% in cardiovascular medicine or surgery units. The indication of danaparoid was evaluated with at least one positive test of heparin-induced thrombocytopenia in eight patients: two patients with both positive platelet and anti-FVII/anti-heparin antibodies test, three patients with positive platelet test and two patients with positive platelet and anti-FVIIa test. The other indications were a previous heparin-induced thrombocytopenia or heparin cutaneous allergy in the past for four patients, cutaneous allergy to heparin during his hospitalization for one patient and clinical suspicion for one patient.

For 13 patients the diagnosis of heparin-induced thrombocytopenia 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 prevent the use of alternative treatments. In patients, platelet count remains difficult among surgery patients, who require a hypocoagulation despite an often low platelet count. In our survey, danaparoid was used in 8 patients, 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.

Methods: We have selected initial chromatographic conditions: separation was achieved with a reversed-phase C18 150 mm (5 mm) and UV detection (λ = 261 nm). The mobile phase consisted of ammonium acetate adjusted to pH = 8 and acetonitrile (54/46, V/V) at a flow rate of 1 mL/min. We have tested many internal standards (Thiopental, Theophylline, Carbamazepine, Butalbital, Indomethacin, and Lidoine) and we opted for Lidoine, because of its good coefficient of regression R = 12.8 and 4.5 minutes respectively. The standards involved liquid-phase extraction of Lidoine and Lidoine based on methanolic extraction of benzene 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 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 (slope) and (intercept). The method was validated according to the French method of imputability of Begaud and all.

Conclusions: 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.

Methods: We conducted an observational retrospective study analysing the medical records of 1180 patients treated by antiplatelet agents and admitted 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 thrombosis.

Results: The annual incidence of haemostasis-related ADR was of 22.7%. Most of them occurred within the first 15 days (70%). Bleeding occurred in 19.2% of the patients (among them 2.54% were severe) and 4.8% of them had a stent thrombosis. Mortality rate was of 1.9% bivariable analysis identified combination of beta blocker, aspirin, ACE inhibitors and statin as a protective factor for bleeding while drug induced abuse (>30 g/days) increases the thrombotic risk. In multivariate analysis anaemia [OR = 2.2 (1.95; 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 in-stent 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.

Conclusions: This observational study demonstrates the correctness of previous studies that 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: Revalvulopathy may be induced by drugs such as 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 patients, a previous echocardiography performed after a mean pergolide interruption of 14 months.

Results: Thirty PD patients treated with pergolide were compared with 30 controls (same mean age 67.8 ± 9.7 vs 62.2 ± 9.7 NS). A pattern of valvular restrictive regurgitation was observed in 1/3 patients taking pergolide (4%). Two patients had heart failure symptoms. Compared to controls, aortic as well as mitral regurgitation appeared to be more frequently observed in PD patients with an odds ratio of respectively 3.1 (95 CI 1.1-8.8) and 10.7 (95 CI 2.1-57). The percentage of frequency of tricuspid regurgitation was not significant.

Conclusion: 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 11 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 (from the one of control group).

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Knowledge 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 Cote d’Ivoire. The questionnaire proposed 16 items with 12 valid questions. The physicians came from different steps of national sanitation system.

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. Those of second line by 46.8%. 2.3% know none ACT and 4.5% could well associate the new policy about fight against malaria.

Results: Thanks: Yannick Arimone, Bernard Begaud and all members of: ARME-Pharmacovigilance Université Victor Segalen Bordeaux 2.

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Heterogeneity of the information for dose adjustment in renal impairment according to the End points characterizations
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Introduction: The use of drugs in patients with renal impairment is based on dose recommendation with strong supportive characterizations (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 (Cr) is only a rough guide for 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 Cockcroft-Gault or 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 incoherencies 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 administered in patients with renal impairment compared with renal impairment (CrCl). Twenty-four cases of bullous drug eruptions associated with autoimmunity diseases in pharmacovigilance unit of Sfax from January 1999 to December 2005. Two methods were proposed to assess the causality of adverse drug reaction. We report 24 cases during 7 years. The causality assessment was treated by two 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 causality relationship between a drug treatment and the occurrence of the 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 (9.3% of all cases). They represented 64% of all cases of adverse drug 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. The main reasons for drug eruptions are: aminoglycosides (19.2%), nonsteroidal anti-inflammatory drugs: NSAIDs (15.38%), sulfonamide antibiotics (15.38%), other antibiotics (11.53%), duretics (11.53%), allopurinol (9.34%) and aspirin (9.34%) and antihypertensive (anti H2) (1.82%).

Conclusion: Practitioners should be informed of this risk and collaborate with pharmacovigilance center. The originality of the new method stems from the use of a logistic model which originality and sensitivity very well the criteria of time to onset.

Keywords: Knowledge – pharmacology – antimalarial drugs - combination therapy.

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Valvular 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. A retrospective study was carried out in pharmacovigilance unit of Sfax from January 1999 to December 2005. Two methods were proposed to assess the causality relationship between a drug treatment and the occurrence of the 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 (9.3% of all cases). They represented 64% of all cases of adverse drug 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. The main reasons for drug eruptions are: aminoglycosides (19.2%), nonsteroidal anti-inflammatory drugs: NSAIDs (15.38%), sulfonamide antibiotics (15.38%), other antibiotics (11.53%), duretics (11.53%), allopurinol (9.34%) and aspirin (9.34%) and antihypertensive (anti H2) (1.82%).

Conclusion: Practitioners should be informed of this risk and collaborate with pharmacovigilance center. The originality of the new method stems from the use of a logistic model which originality and sensitivity very well the criteria of time to onset.

Keywords: Knowledge – pharmacology – antimalarial drugs - combination therapy.

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Bullous drug eruptions and autoimmune diseases
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Introduction: Bullous drug reactions are a rare and severe adverse drug reaction. The association with autoimmune disease seems not to be fortuitous. We report four cases of bullous drug eruptions associated with autoimmune diseases.

Methods: A retrospective study was carried out reporting all cases of bullous drug eruptions associated with autoimmune 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 (16.6%) presented autoimmune diseases: one rheumatoid arthritis, two ulcerative colitis and one nphrotic 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 autoimmune 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 effect. We report a case of valproate associated with psychotropic drugs may cause 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 50 mg/day. Divalproex 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 diazepam 100 mg/day was added. As this latter treatment was initiated on day 4, the 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 umol/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 discontinued, she had 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 metastasis interactions is unlikely because hepatic metabolism of these drugs is not to be different. Although it is possible that pharmacokinetic interactions between valproate and psychotropic compounds have contribute to encephalopathy by addition of their effect on the brain, the hypothesis of an overlap with free drug effects is now 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 sites. 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. This situation should be considered in patients taking drugs which are highly bound to proteins. This emphasizes the potential uselessness of the free fraction determination in therapeutic drug monitoring.

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Automated detection of adverse drug reactions in discharge summaries: a feasibility study
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Introduction: The method of adverse drug reactions detection exist: spontaneous reporting is by far not exhaustive, chart review is very time costly. Analyzing 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 this method to be a reliable basis?

Methods: The study population was inpatients who went out in February 2006 for the second time from March 2003 to March for the second summary. Discharge summary was accessible via a local software developed by the medical information unit for coding purposes. On the first sample, exhaustive human reading was done for the second summary, the cases were detect adverse drug reactions and by the way, to establish a first large keywords list. These keywords were submitted to automated 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, automated 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 described cases of adverse drug reactions were retrieved corresponding to 263 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, sensitivity: 44%, 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: 12%, specificity: 91.6%, positive predictive value: 43.6%, negative predictive value: 84.9%. Age, sex ratio, nature of the reaction, 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 keyword list is adaptable to various studies subjects. It could be optimised by using boolean search software.

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Drug induced hepatotoxicity: an analysis of 570 cases notified to the pharmacovigilance over a 11 years period

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 pharmacological overlap, the cases with elevation of hepatic tests in the absence 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 pains.

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 main aetiology was retained or excluded in 46% of the cases. 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 main aetiology was retained or excluded in 46% of the cases.

Conclusion: This case was notified in regional center of pharmacovigilance of Sfax, Tunisia. An inquiry of pharmacovigilance reaction to both: hypoglycemic sulfonylurea and diuretic sulfonylurea.

Methods: This case was notified in regional center of pharmacovigilance of Sfax, Tunisia an inquiry of pharmacovigilance reaction to both: hypoglycemic sulfonylurea and diuretic sulfonylurea.

Results: A 50-year-old woman had been treated by diltaïzam since 2000 for hypertension, and glibizide, since 2004, for diabetes. In September 2006, she had taked allopurinol for gout. Twenty days later, she had developed hypersensitivity reaction (fever, generalised cutaneous eruption with purpura, and adrenal pain). A biopsy of the skin showed a lichenoid dermatitis and a histological exploration of the skin had been normal. These symptoms were resolved 7 days after stopping all treatment. The challenge of glibizide led to the same symptoms. 10 days after curing, she had taked indapamide and she had developed a generalized pruritis after 1 hour.

Conclusion: The responsibility of glibizide was strongly suspected in the genesis of hypersensitivity reaction in this case. The score of imputability had been evaluated at 15 (CS2) B3 (vraisemblable).

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Sulfonamide cross-reactivity: a case report

Introduction: The cross reactivity between Sulfonamides is discussed. We present a patient who develops hypersensitivity reaction to both: hypoglycemic sulfonylur- ea and diuretic sulfonylurea.

Methods: This case was notified in regional center of pharmacovigilance of Sfax, Tunisia an inquiry of pharmacovigilance reaction to both: hypoglycemic sulfonylurea and diuretic sulfonylurea.

Results: A 37-years-old man treated since 1998 by haloperidol, chlorpromazine and clozapine for refractory generalised epilepsy. He 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 hypothermia, then renal failure and anuria, metabolic and lactic acidosis, rhabdomyolysis. Creatinine 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, oxcarbaepine and pregabalin was started.

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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Cough induced by haloperidol: a case report

Introduction: Haloperidol is a neuroleptic drug. It is an powerful inhibitor of acetylcholine 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 reaction to both: hypoglycemic sulfonylur- ea and diuretic sulfonylurea.

Results: A 37-years-old man treated since 1998 by haloperidol, chlorpromazine and clozapine for refractory generalised epilepsy. He 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 hypothermia, then renal failure and anuria, metabolic and lactic acidosis, rhabdomyolysis. Creatinine 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, oxcarbaepine and pregabalin was started.
This adverse effect had not been described with haloperidol. But the cough reflex seems to be modulated by many receptors: Dopaminergic, serotonergic and sigma receptors.

**Conclusion:** When patients consult for cough, clinicians should think about drug induced asthma, particularly when other aetiologies are eliminated.

### 300 Fluoroquinolone psychiatric adverse effects: review of French pharmacovigilance data base

**Introduction:** Psychiatric adverse effects of fluoroquinolones are known for a long time. The mechanisms are still matter of debate.

**Objective:** We reviewed the available literature to draw conclusions about the risk of fluoroquinolone-induced psychiatric adverse effects and to discuss the mechanisms of these reactions.

**Methods:** A Medline search was conducted with the terms “fluoroquinolone” and “psychiatric adverse effects” and a “French pharmacovigilance data base” search was performed. All adverse effects were collected.

**Results:** A total of 258 cases have been reported concerning 142 women and 116 men. Mean age was 58 years, and 39% were above 65-years old. For each report, the gender, the psychiatric adverse effect, the seriousness, the time of onset and duration, fluoroquinolone involved, dose and evolution were recorded.

**Conclusion:** Despite methodological biases were identified, the questionnaire's replies and comments proved the needs of knowledge on risks and vigilances. It connection for all hospital staff.

### 301 Analyze of users’ expectations of a hospital network system in risk management

**Introduction:** The risk management is not only necessary but mandatory for all health care authorities organized vigilances on nosocomial diseases, drugs, medical devices, practitioners. Hemovigilance, materiovigilance, pharmacovigilance and nosoco-miovigilance are well identified. New vigilances or specific target so as 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 preclude the possibility to identify the class of drugs (11%). The avoidable adverse effects are more often due to an error of the patient (53%). 33% seem to be related to the medical prescription and 5% on the pharmacological act. Lastly, 7% remain unspecified and 17% non-informative.

**Methods:** A questionnaire was sent to the medical and paramedical users identified in the computerized mailing lists of the hospital. The identification of professionals' experience was considered a proxy for the knowledge. The questionnaire’s terms were adapted to the needs on risk and vigilance of 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 midwives. 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 least by practitioners. Hemovigilance, materiovigilance, pharmacovigilance and non-miovigilance are well identified. New vigilances or specific target so as 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 preclude the possibility to identify the class of drugs (11%). The avoidable adverse effects are more often due to an error of the patient (53%). 33% seem to be related to the medical prescription and 5% on the pharmacological act. Lastly, 7% remain unspecified and 17% non-informative.

**Conclusion:** Despite methodological biases were identified, the questionnaire’s replies and comments proved the needs of knowledge on risks and vigilance of the responsible persons and to contact them were analyzed. The whishes and expectations of the users were collected.

### 302 Antipneumococcal vaccine: inefficacy in a splenectomised patient

**Introduction:** Antipneumococcal vaccine: inefficacy in a splenectomised patient

**Methods:** A breast carcinoma patient was treated by a 39-year-old woman had a breast carcinoma. She was treated by phenobarbital (10 cg daily) since 20...
Conclusion: Practitioners prescribing phenobarbital should be informed of this risk and should inform pharmacovigilance center if such symptoms appear.

205 Cohort study of serious adverse side effects after influenza vaccination in old people’s home in the departments of Loire Atlantique and Vendée C Cohen, B Uzzan, A Dumont-Fischer, D Dumont-Fischer, N Lahhou, MC Boissier, JC Alvarez, GY Perret, B Cohen, MA Hugues, B Ayet, F Frangeul, FFranc, Y Legue`, GY Perret

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 (edema, erythema, pain and induration) or general reactions, like fever, discomfort, shivers, muscle and articulation pain. These side effects are frequent in the elderly. 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 (Absaps). However no causal relationship between adverse events and the vaccine was proved.

Methods: Nurses, qualified professionals or physicians of 177 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 sent 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 than 90% have got vaccinated, the others have refused vaccination or used homeopathy vaccination. Only elderly individuals have got vaccination and only 3% of them are connected to the pharmacovigilance program in our region of Nantes. Among these reports, only four have concerned a serious adverse side effect (death, acute pulmonary edema, 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 cardiovascular diseases and for lower respiratory tract diseases, reduced risk of respiratory tract infection 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 vaccination 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 immunisations are an important part of their care.

306 Increased serum testosterone related to phenylbutazone therapy: a drug-hormone interaction

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, Bobigny - France) in a 30 year old man with a serum testosterone of 38.7 nmol/l (TRACE) 7 days after influenza vaccination. Two other patients without spondylarthritis. His gonadal function was clinically normal. He denied any intake of antiandrogen or antiestrogen therapy, but his medication comprised several testosterone assays, or elsewhere an interference of phenylbutazone with the tracer. This drug-hormone interaction might be a common epitope shared by phenylbutazone and oxyphenbutazone.

Conclusion: Increased serum testosterone related to phenylbutazone therapy: a seemingly rare drug-hormone interaction.

Methods: We used other testosterone assays (DLS RIA, Webster, USA and BRAMHS TRACE on KRYPTOR automatic, Berlin, Germany) than in the previous publication, to exclude an exogenous source of testosterone and the inhibition of cell-mediated immunity, allowing for the development of invasive bacterial or fungal infections.

Bacterial or fungal infections. 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 two fatal lesions in two patients with chronic analgesic use.

Methods: These two cases were notified to the evaluation and information on the French pharmacovigilance centre.

Inhalation of heroin in two patients with chronnie analgesic use.

Case 1. It concerns a 36-year-old man with a history of 6 years heroin nasal inhalation only. In November 2006, he consulted the emergency unit group of thrombotic complications 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 arm and intravenous heroin was stopped. In October 2006, she complained of pain from nasal pain. At the exam, nasal septum and sinusosal wall necrosis with suppurating rhinorhoea 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 oxycotin. The mechanism of tissue necrosis with opioid abuse remains unknown. One possible explanation may lie in the effect 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.

309 Prospective clinical and biological follow-up of three breastfed babies from hydrocodeine-treated mothers
B Uzzan, F Gregoire, A Pariente, F Haramburu, N Moore

Introduction: Adverse events 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. 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 putative 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. We wanted to study the potential effect of confounding on automated safety signals generation in a situation where no putative effect would be expected.

Results: There was no association between angiotensin II receptor antagonists and other drugs and hypoglycaemia was also tested in the subgroups of patients taking or not ACE inhibitors.

Conclusion: There was 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 but could be suspected disappeared after stratification on antidiabetic agents use, thus demonstrating the role of confounding by indication in its generation.

308 Necrosis of the intranasal structures and soft palate as a result of nasal heroin inhalation

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 two fatal lesions in two patients with chronic analgesic use.

Methods: These two cases were notified to the evaluation and information on the 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 pharmacy.

Result: Case 1. It concerns a 36-year-old man with a history of 6 years heroin nasal inhalation only. In November 2006, he consulted the emergency unit group of thrombotic complications 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 arm and intravenous heroin was stopped. In October 2006, she complained of pain from nasal pain. At the exam, nasal septum and sinusosal wall necrosis with suppurating rhinorhoea 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 oxycotin. The mechanism of tissue necrosis with opioid abuse remains unknown. One possible explanation may lie in the effect 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.
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 CYP2T14 gene was determined only for the babies 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.

Conclusions: In three cases, breast feeding 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.

310 Colchicine-induced pancytopenia during therapeutic dose administration. French pharmacovigilance database survey and literature review

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 (62 mg/d).

Methods: All case-reports of pancytopenia and narrow depression collected in French Pharmacovigilance database between 1984 and May 2006, and from literature were analyzed.

Results: Forty-two case-reports were retrieved: 33 from database and nine from literature.

Conclusion: Colchicine can be toxic even at therapeutic dose. It should be used with extreme care in patients receiving CYP2A6 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-licence regarding this risk.

311 Pharmacovigilance in clinical trials: a tremendous change

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 immediately inform the competent authorities in case of a serious adverse event and assess the causality of the event. The sponsor now becomes 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 and analyse all 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 also assess the causality and the expectedness of these adverse events.

Results: Sponsors must report electronically SUSARs to both EMEA through the European pharmacovigilance database EudraVigilance, the AFSSAPS and the relevant Ethics Committee. 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 communicated to the sponsor.

Conclusion: New regulations will better protect the rights, safety and well-being of patients taking part in clinical trials of medicines. Moreover, enhanced vigilance 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.

312 Choice of the comparator for signal generation in pharmacovigilance databases: impact on detection thresholds

Methods: 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 other drugs.

Results: 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 (62 mg/d).

Conclusion: Colchicine can be toxic even at therapeutic dose. It should be used with extreme care in patients receiving CYP2A6 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-licence regarding this risk.
drug reaction (ADR). Several cases of hyponatremia in old people taking thiazide drugs and invited to drink 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, who had a level with heat wave preceding the occurrence, 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). Sex rate (73.8% vs. 78.5%) and age ratio (0.98 vs. 0.8) were comparable. During 2003, 73 drugs [58 possible (21), 12 plausible (12) and three likely (3)] were involved. During 2006, more plausible cases were notified [57 (11)], 24 T2, and 12 (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 (SRI) (16.8% vs. 7.9%), NOX, thiazide and thiazide-like diuretics, ACE and propranolol pump inhibitors (5.8% and 5.2%, NS). Clinical outcomes were similar in 2003 and 2006 (mainly favourable).

Conclusion: The main 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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How to identify rare adverse drug reactions for pharmacogenetic studies: example of Torsade de Points in France
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Toulouse - France
Introduction: Drug-induced Torsade de Points (Tdp) is one of the most serious adverse drug reactions (Adr) 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 case setting, this prolonged QT interval is directly attributable to medications the patient is taking. These Adr are 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 infaraction, or known electrolyte disturbance. We searched in the French Pharmacovigilance Database between 2000 and 2005 cases with terms ‘Torsade de points’ and ‘prolongation of QT’.

Results: 97 files were examined, 64 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 live patients from Pharmacovigilance database.

Conclusion: The two systems are very effective to identify patients with very rare Adrs for the EUDRAGENE project. The most serious ADRs are notified and more patients are dead in Pharmacovigilance database. EUDRAGENE project 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
P Ollivier, JL Montastruc, Y Mouget - Toulouse - France

Introduction: Patients suffering from osteoarthritis (OA) are often tempted to try long acting drugs like chondroitin sulfate, diclofenac or avocado-soybean unsaponifiables (ASU). 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 Presso.

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 ASU). Adr with ASU were more frequent for long acting drugs (15% of cases reported) notified (mostly hematological 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. Other significant ADRs were: coagulation and platelet 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, increase 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 under-notification). These safety data should be discussed with the poor expected clinical benefit of ASU in rheumatology.

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www.e-lactancia.org: internet source on breastfeeding, drugs use and abus substances
M Parti, T Taliana, L Landa Rivera, B Besler Soto, MJ Benlloch Munchrz, M Sanchez Palomares, L Santos Serrano, A Sendra Mengual, M Ferriol Camacho, M Buigues
*Denia (alicante) - Espagnè; ¹Denia - Espagnè

Introduction: Paramount importance of breastfeeding is 90% of women who breastfed get medication. Misinformation on the compatibility of drugs, phytotherapics and breastfeeding can lead to withdrawal unnecessarily. A web source with information on compatibility of medication, other substances and breastfeeding.

Methods: Seud 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 of tablets to be taken (ii) formula choice. 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 dietetic choice.

We have 1470 products listed at four lactation risk categories:

<table>
<thead>
<tr>
<th>Category</th>
<th>Risk, problem</th>
<th>Breastfeeding compatibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>0/Green</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>1/Yellow</td>
<td>Mild</td>
<td>Yes</td>
</tr>
<tr>
<td>2/Orange</td>
<td>Moderate</td>
<td>Assess risk/benefit</td>
</tr>
<tr>
<td>3/Red</td>
<td>Severe</td>
<td>Assess risk/benefit</td>
</tr>
</tbody>
</table>

*Abras 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 breastfeed-
ing.

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Misused of medicines in urban and festive areas
JH Bourdon, J Arditti *Marseille - France

Introduction: An evaluation of the nature of the psychoactive substances misused in urban and festive areas seems to be very difficult. The EUS (European system of Toxicological and Substances) of the CSF (Centre of Information on the Lipids of the substance) is a medical emergency unit system. This evaluation, made by the CSF (Centre d’Evaluation et d’Information sur la pharmacodependance) system, allows to identify numerous medicines sold as ecstasy pills.

Methods: Samples (pills, powders, capsules, liquids…) were collected by the SINTES device (Systeme d’Identification National des Toxiques et Substances) tool for the French monitoring centre for drugs and drug addiction (FMCD). A physical description and a photography were performed before analysis. Immunochromes researches are practiced in order to find stupifying molecules as amphetamines, cocaine, cannabis and opiates. Complementary analysis with chromatochromatography mass spectrometry techniques allows to identity the different components.

Results: Analysis lead to discover numerous molecules of medicine. Three main groups are 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 specialties with attractive logos are various like bullomedil, chloroquine, metamizol, flocetamine. The molecules used for dilution are chloroquine, lidocaine. For some medicine, the galenic aspect varies, for example for chloroquine which is found as pills, but also as powder for used for diluting cocaine.

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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Myocardic 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 this hemopathy has been recently improved with the development of new drugs.

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. Echocardiography 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 dyspea 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 4 months 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 chronology, (ii) patient with no cardiac history, (iii) case reports of cardiac adverse effects with thalidomide, a parent compound of lenalidomide. Dysrythmia (essentially bradycardia), myocardic 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 anonymised form, approved by the Council of Forensic Science in 2001, is filled by the expert: individual characteristics, history of abuse, circumstances of the fatal abuse, and toxicological analysis results (substance identification and blood concentration).

All forms are transmitted to the CEF (Centre d'Évaluation 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, delirium tremens, accident in subway). 80% of men, average age of 31 years, illicit drugs, mostly heroin and cocaine, were involved in the majority of the case series (48 of 65–73.5%). Opium substitution treatments were at the origin of 10 deaths (eight cases with methadone). Other legal opiates medicines (codeine and morphine) induced 10% of the reported deaths. Polytoxicomanias (alcohol and/or cannabis found in one third of the case series) and associations with legal psychotropics (43%), mainly benzodiazepines.

**Conclusion:** DRAMES is one of tools to evaluate the possibility of abuse of psychoactive substances. Its data are used by the French Ministries of health and Interior. Despite some improvement concerning drug-related death cases, are still studied: improvement in the number of 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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**Hypotenatremia 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 hypotenatremia. Many 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 factor medication or ancillary on occurrence of ISR.

**Methods:** A retrospective study was undertaken at Toulouse University Hospital Trauma Center 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 reviewed. Patients undergoing treatment with clopidogrel (75 mg/w) or 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 stenting. Group 1 included patients by their resistance 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: 65.6 ± 11.8 years) with an ISR, 20.2 ± (17.0) years follow up (group I) vs 69.0 ± 10.7 years follow up (group II) and 35.7 ± (10.0) mg clopidogrel/d day after PCI. Group II received more dose of clopidogrel compared to group I (143 mg ± 30 vs. 90 ± 30 mg, P < 0.0001). We did not find any significant difference between the two groups concerning length, type (drug-eluting stent or not), number of 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.
revealed concerning right lung, causes anomalous 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 levocetirizine and topiramate in the occurrence of these findings because 4 other etiologies 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.

329 Accidental child poisonings with cannabis derivatives reported to Lyon Poison Centre between 1999 and 2006

**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:** 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 guilt 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 bradyphrenia have nonetheless been reported in some cases.

**Conclusion:** Cannabis derivatives are a potential, even though still uncommon cause of acute poisoning in toddlers. Surprisingly it was raised in 23% (24/105) of the cases presenting with reduced consciousness of unknown cause, ataxia, mydriasis, bradyphrenia. An analytical screening is advisable to rule out other toxic agents.

330 An automated method to eliminate bias induced by co-prescription in safety signal generation using spontaneous reporting database

**Methods:** An automated method is based on a backward stepwise procedure for the elimination 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 OR and an IC% 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 using the OR of the association of the 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 on the French pharmacovigilance database involving 16 years of data (1990–2006).

**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, aminopterin, februbamine, phenobarbital, metformin, pravastatin, digoxidosterone and dapsone. Signals that were initially generated in the whole database and that no longer appeared using this method concerned progabide, topiramate, clozapam, iron and vitamins, vigabatrin, clonazepam, lamotrigine, amphetamine, diazepam and bufotenine.

**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 cases specific to pharmacologic epidemiology, such as channelling due to co-prescription.

331 Drug interactions with cholinesterase inhibitors: an analysis of the French Pharmacovigilance database

**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 reports 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 and forward searched. National Formulary (Vidal), National Resource Institute (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, an estimated summary 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 (8.3%) cases. Dapoxetine was 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 abundant for ChEIs and occur 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.

332 Hyperesthesia response to phenobarbital in a fat patient

**Methods:** A 19-year-old man, weighing 110 kg and heighting 1.8 m. Operated at January 2000 for a benign cerebral tumor. This patient had been treated from 01/15/1995 with phenobarbital, 60 mg tid. Three days after surgery, the patient presented with increased pain. Then after this treatment, this patient developed a generalized erythematous maculopapular plaque, as well as multiple vesicle and pustule associated with facial oedema, oral pharynx.

**Results:** Face to these clinical features, phenobarbital was withdrawn and replaced by phenytoine since 02/02/2000. The patient has received also local symptomatic treatment, at 04/04/2000 the patient presented with pain exacerbation and at this date, phenobarbital level was 9.62 mg/L. A week later (02/21/00) the pustulous lesions disappeared, oedema, pruritus 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.

**Conclusion:** Hyperesthesia response induced by phenobarbital with slow amelioration (6 weeks) and lesions extension after drug withdrawal. The clinical presentation was confronted with determining of phenobarbital plasma level.

333 Tipranavir French cohort ATU: safety results from 295 patients

**Methods:** 18 July 2005 to 15 March 2006, 295 triple class experienced patients were entered in the cohort ATU and were treated with TPV/500/200 mg bid. According to the cohort ATU protocol and AFSSaPS requirements, only adverse drug reactions 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.

**Conclusion:** No serious AEs were reported. One patient (0.3%) reported serious bleeding event. The overall incidence of SAEs was 21.3 per 100 patients exposure year. In total, 5 patients (1.7%) experienced nine SAEs that were not related to the treatment, five skin bleeding were reported.

334 A Pariente, N Moore, F Haramburu, A Sommet, F Thiessard, JL Montastruc, CH Belkahia, F Miremont-Salamé, A Foureur, M Fourrier, M Montpellier - France

**Methods:** A Pariente, N Moore, F Haramburu, A Sommet, F Thiessard, JL Montastruc, CH Belkahia, F Miremont-Salamé, A Foureur, M Fourrier, M Montpellier - France

**Conclusion:** Phenobarbital responsability was retained because: phenobarbital was the alone drug taken 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 due to low hepatic and kidney adsorption followed by patient corpulence, and release of this drug in plasma controlled by phenobarbital monitoring.
Conclusion: During the French TPV cohort ATU, analysis of collected AEs did not reveal any significant change in frequency despite surgery known AEs for TPV confirming the favourable safety profile of TPV in current practice.

334 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 legals 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: 18 months; range: 5% after treatment withdrawal; mean: 23 months). Endoscopic diagnosis conducted in four cases revealed inflammatory colitis. Recovery was observed in five patients. Relapse was possible in two cases. Reaction led 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.

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) is general and has been reported to aggravate preexisting IBD; so careful consideration about isotretinoin treatment should be made in patients at higher risk for IBD.

335 Drug-induced dementia: a case/no-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 used the case/no-case methodology. Cases were reports of dementia, worsened dementia or dementia 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 more than 2-years old), a clinical consultation or a dossier could be done. Thirty percent were children (less than 12 years), 1% were women during foetal period. Among the six other cases that are HIV positive, only one was treated during foetal period. However all of these six newborns had sequelae, 5% totally recovered, the outcome being unknown in 10%.

Conclusion: Drug-induced dementia is a serious adverse effect. Children born from HIV positive mothers is an important public health issue. Knowing the drugs that are involved is crucial for health care providers.

336 Does drugs without adverse effects really exist?

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*Montpellier - France

Methods: We analysed all cases of necrotizing colitis during neuroleptic therapy notified in 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, neurological disorders, 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%.

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 antiinflammatory drugs. Close monitoring of digestive functions seems required to reduce the number of associated anticholinergic drugs and administration of laxative therapy.

337 Oligoelements: 16

Eye drops: 15

Vitamines: 19

Phytotherapy: 52

Topical drugs: 24

Conclusions: So it seems interesting to classify these products according to presentation and use. Topical drugs: 24

Allergens: 1

Vitamines: 19

Eye drops: 15

Venotonic drugs: 7

Oligoelements: 16

Allergens: 1

Conclusion: 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 I% of drug allergy. Bihanol was found in 30 cases, which is not a problem in normal use but accidental children ingestion should be worrying. Aroma: 24, 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 1, one food red 14

Terpenoids: 15 Preservatives: 16 especially with eye drop.

Conclusion: In the French pharmacovigilance data bank we find with these 228 drugs and in particular this prophylaxis we should take some in consideration. When the adverse effect take place it seems interesting to detail 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.

338 Children born from HIV + mothers at Clermont-Ferrand and Saint-Etienne:

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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 accomplish 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 child.

Results: Eighty-two couples of mother-child were identified. Thirty-six mothers and one father 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 for mother during pregnancy 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 first period.

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-listening problems in the Emergency Department in France. The objective of this study was to document and 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 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).

**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 1:1.68.). ADRs were reported in 40 patients (>70% of the patients). Patients aged from 40 to 49 years ranked in second position (16%). Drugs used were mainly benzodiazepines (26%) and antidepressants (12%). Concomitant acute alcohol exposure was identified in five cases. The most frequent ADR was gastro-intestinal disorders. The system organ the most often reported was nervous system disorders (25%), followed by blood (20%) and cardiovascular (18%) 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 implicated 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 among aged 40–49 years represents a population at risk for ADRs. Monitoring of ADRs reports from ED could provide useful information about pharmacocopeiody of outpatients ADRs.

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**Mesalazine-induced oligoamnios: first case report**

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**Introduction:** More than 300 cases of women treated during pregnancy with mesalazine are available without any increase of congenital abnormalities risk or fetofoetal 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 ultrasound showed signs of oligoamnios. At 35 weeks gestation, the oligoamnios is confirmed at 35 mm. The fetus was eutrophic and foetal kidneys were without abnormalities. The cardiac rhythm was correct. Unilateral, cerebral and uterine arteries Doppler were normal. A premature membrane rupture was excluded. As no maternal pathology (infection, arterial hypertension) was diagnosed, the response of mesalazine was confirmed at 35 mm. The fetus was eutrophic and foetal kidneys were without abnormalities.

**Conclusion:** No abnormalities were reported in the newborn. Parents and professionals implied. It would be more than advisable to put in place important steps to ensure the safety of the mother and the birth of the newborn in case of gestation shows normal amniotic fluid level (100 mm).

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**341**

**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 the short and long-term consequences of episodes of attempted suicide during pregnancy and analyse the outcome of pregnancy.

**Methods:** Requests received from January 1994 to December 2004 by both Lyon Poisson and Clermont-Ferrand Centre of Pharmacovigilance were analysed. Information on the patient, details on acute drug exposure, and management of poisoning were collected at the time of the initial inquiries. The outcome of pregnancy was the primary objective and was assessed prospectively.

**Results:** A cohort of 237 pregnant women who attempted suicide with medicines during pregnancy is described. Their mean age was 37.1 ± 6.6 years. One 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 3rd and 5th month of pregnancy in 13 (55.5%). Among 112 newborns, in 38.8 weeks and 16 neonates were premature (14.4%, 95% CI: 8.5–22.4%). A meconium stained amniotic fluid was observed in 10 cases (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 polynamolization 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 tends to show 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**

Cerveny 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, fibrinolysis (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. 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). 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). 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).

**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 an allergology consultation. Only 44% of the 32 anaphylactic reactions of the survey were explored in allergo-anaesthesia consultation. On the 62 medicinal shocks, only eight of them were declared in Pharmacovigilance (13%). 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. With chronic diseases such as asthma, chronic obstructive pulmonary disease, among the ones where responsibility was shown by cutaneous tests or IgE specific tests in allergology, they account for only seven cases. However, among suspected but not established diagnosis, the most incremented substances are the ones used in anaesthesia and latex, a total of 20 of the 46 cases in this category.

**Conclusion:** Non-respect of these rules of practice about allergology consultation and pharmacovigilance declaration appears to be very frequent. We hope this survey will reach to a dialogue between the different health 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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**343**

**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 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 (2.5, 5.0, 10.0 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 cell infiltration and interstitial angiectasis of cardiac muscles; in the liver there was liver cell regeneration, point necrosis and liver tissue inflammatory infiltration. There was severe concomitant lesions of the cell organelles on electron microscopy. These ultrastructural changes were significantly improved by ASM.

**Conclusion:** This model of traditional Uyghur medicine syndrome of abnormal savda is associated with morphological and ultrastructural damage of the target organs. It is recommended to proceed to an allergology consultation from 6 to 8 weeks after the shock. As well, fibrinolysis (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.

**Conclusion:** This model of traditional Uyghur medicine syndrome of abnormal savda is associated with morphological and ultrastructural damage of the target organs.

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**Cardiac tolerance towards antipsychotic combinations**

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**Dijon - France**

**Introduction:** Sudden deaths have been reported in patients receiving psycholeptic combinations and the early anti-psychotic combination to be more frequently associated with a prolongation of the QT interval. Increasing length of the QT interval can degenerate into torsades de pointes and the combination of drugs
increase the risk of sudden death. As precaution, in 2003, based on literature, the French agency for the sanitary security of health products (AFSSAPS) published a list of twelve antipsychotics (chlorpromazine, cyamemazine, levomepromazine, thioridazine, trifluperazine, sulpiride, sulphopride, tiapride, droperidol, haloperidol and pimozide) whose association is ill advised (and contraindicated with thioridazine) to the clinical research. Other events were related to the

Methods: The study was carried out during a 16-month period among patients hospitalised in a geriatric 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 inadmissible combination of antipsychotic, patients with combination that was inadmissible, patients who had not received any antipsychotic (control group). We compared in an univariate study QTC value difference of the three groups using the non parametric Mann–Whitney. Then, different factors involved in the lengthening of QTc where included in a multivariate analyse.

Results: Nineteen clinical studies were concerned, mainly involving drugs (63%) 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 analysed.

Results: We found 71 case reports of adverse drug 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 65.7 ± 11.8 kg, number of daily drugs 4.45 ± 2.04. Most of patients suffered from polypharmacy (57.7%). More than a half side effects were serious (62%), with one third concerning cardiac function, and one third central nervous system. A drug-drug interaction was considered in 36% of cases. There were 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 (81.8 ± 5.1 vs. 78.2 ± 4.5 years, P = 0.037), 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 acetylcholinesterase inhibitors, especially cardiac and neurolgic. 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.

Conclusion: This study underlines the importance of SAEs for an institutional concern. Further clinical studies on the impact of these events on functional status and on cognitive impairment are needed to determine the necessity for institutional promoters to involve regional pharmacovigilance centers in this activity.

347 The use of levonorgestrel, an emergency contraception method, investigated in a community pharmacy


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 includes alternative treatment when a risk of unwanted pregnancy is suspected. Using reliable information technology, it was observed an abnormally high dispensation of Levonorgestrel after pill" in 2005. That is why we decided to us this 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 2/3 any non-user to whom this product was dispensed (sexual partner, 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 percent of users were minors and 60% were 20–39 years. The main sources of information about the availability of the contraception method were the friends 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 several times and 8 times. No one asked for more than 72 hours before a sexual intercourse. In 53% 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. 93% of seekers were aware that there is a risk of pregnancy occurrence of the method. In 17% of users didn't know that they had to take 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. 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 not to 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.

348 Prevalability of adverse drug reaction in elderly with dementia


Introduction: Iatriogen pathogenic is known to be frequent in elderly, prevenant 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 suffering from cognitive impairing diseases or related to the study. Eighty-two patients, mostly women (73%), aged 87 (±4.9) years with cognitive impairment (mini mental status ≤ 15± 5.7) were included in a short geriatric unit of a French university hospital. Eighty-two patients, mostly women (73%), aged 87 (±4.9) years with cognitive impairment (mini mental status ≤ 15± 5.7) were included 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 2/3 any non-user to whom this product was dispensed (sexual partner, family member, neighbor, colleague). The survey was given by the same pharmacist.

Results: Eighty-two patients, mostly women (73%), aged 87 (±4.9) years with cognitive impairment (mini mental status ≤ 15± 5.7) were included in a short geriatric unit of a French university hospital. Eighty-two patients, mostly women (73%), aged 87 (±4.9) years with cognitive impairment (mini mental status ≤ 15± 5.7) were included in a short geriatric unit of a French university hospital. Eighty-two patients, mostly women (73%), aged 87 (±4.9) years with cognitive impairment (mini mental status ≤ 15± 5.7) were included in a short geriatric unit of a French university hospital.

Conclusion: Thus, a preventability of risk of adverse drug reactions exists in elderly with dementia related to under-evaluation of dementia. Therefore, search for improve in this domain is of great importance, to provide some support to compensate or to provide an adequate drug utilisation in this population.

349 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/C/D 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 alphabetic types, including C ("catastrophic") and D ("delayed"). The purpose of our study was to apply the A/B/C/D alphabetical classification to ADRs notified to the French Pharmacovigilance centre and then discuss the difficulties to apply it.

Methods: A total of 2117 ADRs notified during the 4-month period (November 2004 to February 2005) and, among them, selected those with causality assessment L2/L2 "plausible". For each pair "drug/ADR", we analysed drug information (pharmacological class, respect of dose or not, causality assessment), clinical information (class-organ, time to onset, possible risk factors, severity, outcome, and impact on disease management). At the following consultation, they completed the questionnaire with the help, if necessary, of our team. Clinical or biological data were also obtained for all severe or life-threatening events.

Results: Among the first ADRs notified in 2005, 67 were classified in type A, 24 in type B and two in type C. A total of 147 encountered 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 anti-infectious. Type B reactions concerned cutaneous, hematological and neurosensory 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.

350 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 2 intravenous doses administered 3 mg/m2 at a 48-hour interval. 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 for the treatment of relapsed or refractory multiple myeloma. Each course of treatment consists of 2 intravenous doses administered 3 mg/m2 at a 48-hour interval. 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 for the treatment of relapsed or refractory multiple myeloma.

Methods: In this prospective real-life survey, serious adverse effects were reported in 80 patients (78%). The 23 remaining patients were considered to be well tolerated compared to standard chemotherapy. The most frequently reported ADRs were drugs acting on central nervous system (analgesics, antipsychotics) and anti-infectious. Two ADRs have been classed in type C.

Results: Over the 7-month period, 14 patients received a total of 61 courses (225 drug administrations during the fourth infusion). Clinical trials have shown 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 for the treatment of relapsed or refractory multiple myeloma. Each course of treatment consists of 2 intravenous doses administered 3 mg/m2 at a 48-hour interval. 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 for the treatment of relapsed or refractory multiple myeloma.

Conclusion: In this prospective real-life survey, serious adverse effects were reported in 11 of 14 patients (79%), an incidence higher than in clinical trials. In patients because of peripheral neurotoxicity. In addition, further bortezomib treatments were required at higher dose (mean value 5.9 ± 2.8 drugs) as follows: prednisone (48.5%), mycophenolate mofetil (30.5%), reombinant erythropoietin (15.3%), alpha interferon (9.3%), G-CSF (5.9%), azathioprine (5.9%) proton pump inhibitors (30.5%) and statin (28.8%). A total of 1.189 [neurological (30.1%), cutaneous (12.2%), hematological (12.4%), osteo-sclerotic (10.1%), gastrointestinal (8.2%), and various (14.9%)] ADRs were collected. Multivariate analysis showed that tacrolimus-induced tremor occurred more in patients recently transplanted (<1 year) related to high tacrolimus serum concentration. 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, hirudination and gingival hypertrophy was significantly higher (P < 0.0001) in patients exposed to ciclosporin for more than 7 months. This survey underlines the interest of ADRs' self reporting by patients in order to improve the collect of ADRs non serious but nevertheless important for patients' quality of life.

352 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 immuno-suppressive 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, and presence of any ADR. At their visit, they completed the questionnaire with the help, if necessary, of our team. Clinical or biological data were also obtained for all severe or life-threatening events.

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 66 ± 36 months (0.5–252). The transplant was performed for end-stage hepatic cirrhosis (11.6%), hepatitis C virus (HCV) infection (31.6%), hemochromatosis (8.8%), primary biliary cirrhosis (PBC) (7%), hepatitis B virus (HBV) infection (7%) and alcoholic liver hepatitis (3%). Corticotherapy did not increase significantly 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, hirudination and gingival hypertrophy was significantly higher (P < 0.0001) in patients exposed to ciclosporin for more than 7 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 important for patients' quality of life.

353 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) gene coding for VKORC1 has 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 VKOR1 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. VKOR1 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 VKOR1C1 TT genotype might be associated with lower risk of VTE. 

Results: The relationship between VKOR1C1 and VTE seemed 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 VKOR1C1 TT genotype might be associated with lower risk of VTE. 

Conclusion: In this case-control study, VKOR1 C1 allele is associated with a reduced risk of venous thromboembolism. Other investigations are required to precise underlying mechanisms.

354 Hormone therapy and risk of venous thromboembolism among postmenopausal women. Impact of cytochrome P450 3A5 genetic polymorphism

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Introduction: Oral estrogen use increases the risk of venous thromboembolism but conflicting results have been observed. We investigated the association between VKOR1C1 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. VKOR1 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 VKOR1C1 TT genotype might be associated with lower risk of VTE. 

Results: The relationship between VKOR1C1 and VTE seemed 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 VKOR1C1 TT genotype might be associated with lower risk of VTE. 

Conclusion: In this case-control study, VKOR1 C1 allele is associated with a reduced risk of venous thromboembolism. Other investigations are required to precise underlying mechanisms.
hepatic catabolism of steroidal hormones, especially estrogen. Subjects with the CYP3A5*1/*1 and CYP3A5*1/*5 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 successively evaluated in 153 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 = 1.8; 95% CI: 2.1–6.5) 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 patients without CYP3A5*1 allele and 22.1 (95% CI: 3.7–113.2) 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)**


**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 alf-a2a (40 kDa) 180 μg/week and ribavirin with dose adjusted on body weight (<75 kg, 1000 μg/day, >75 kg 1200 μg/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 >2log10 over the first 4 weeks of therapy (Real-time PCR assay, HCV Ampliprep® Taqman).

**Results:** High inter-patient ribavirin AUC 0–12 h variability was found (range 1.266–6916 μg/L/h), 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 (3451 ± 1378 vs. 3025 ± 1030 μg/L/h, 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%: 0.586–0.930, P = 0.0038). A ribavirin AUC 0–12 h ≥ 3128 μg/L/h 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 1A4 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 of atherothrombotic complications. Clopidogrel is a pro-drug requiring a metabolic activation which is mainly mediated by cytochrome P450 2C19 (CYP2C19). Recently, two common polymorphisms in genes encoding for the CYP2C19 (G681A, allele-*2*) and CYP1A4 (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 aggreometry 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. 61.2% (IR: 19.1–81.6) 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, hematomorcy, collagen lag time, and the fibrinoen 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.
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) patients with moderate to high disease activity using methotrexate (MTX) concomitantly.

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 = 196) without MTX (n = 186). 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-S3435T (*3, n = 380), MDR1-S69T (n = 377), MTHFR C677T (n = 180) and MTHFR A1298C (n = 180). For each gene, alleles and genotype frequencies were calculated at baseline and with ACR 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 = 0.0035-0.76 (95% CI 1.13-2.71)). Response to adalimumab was similar between patients treated with or without concomitant MTX (P > 0.05). The MDR1-S3435T *3 allele correlated with ACR50 response (P = 0.035). The median daily doses of TAC per body weight of TAC to obtain the target trough concentration were respectively at baseline 100, 1.65 ± 1.0 mg/kg/d and 180 mg/kg/d for CYP3A5 1/*1, 1/*3 and CYP3A5 1/*3 genotype (P = 0.0049). Similar results were obtained at 1 year post transplantation (P = 0.0068), MDR1 C3435T polymorphism was not related to ACR pattern of response and TAC daily dose requirement.

Conclusion: CYP3A5 genotype and ACR pattern significantly influence cyclosporin 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.

Study of the G-protein beta 3 subunit gene (C825T) polymorphism and olanzapine or risperidone – related weight gain in patients with schizophrenia.

Background: Several studies have indicated that MDR1 and MTHFR non-MDR1 gene polymorphisms in patients co-treated or not with MTX are predictive of response to anti-TNF therapy in RA patients. ACR50 pattern of response was also similar regardless of genotype: MTHFR C677T (G/G, 40%; G/T + T/A 42%; T/T + A/A 36%) and CYP3A5 S3435T (n=380). For each gene, alleles and genotype frequencies 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.

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Conclusion: CYP3A5 genotype and ACR pattern significantly influence cyclosporin 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.
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, 354 and 2677 genotypes (all P values >0.05).

Conclusion: Results suggested that MDR1 C1236T, G2677T and C3543T polymorphism could 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 transcription activity

Introduction: The α2B-adrenergic receptor is expressed in blood vessels, and is responsible for the transient vasoconstriction following α2-agonist administration. Previously we 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 HaCaT).

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 mechanisms of this effect, and to delineate their possible impact on α2B-adrenergic receptor expression in vivo.

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Anti-inflammatory effects of formoterol in rats after a single inhalation of nebulized caco7 cells

Results: Formoterol significantly attenuated scores of lung lesions associated with parenchyma inflammatory cell infiltration and congestion observed in CgD group. It elicited a dose-dependent significant decrease in the number of CD45+ cells in a dose-dependent manner (number (8.6 ± 10^6 ± 4.1 (higher dose) vs. 23.2 ± 7.7 ± 10^6 cells/ml; P < 0.001), neutrophils (2.3 ± 0.6 ± 10^6 vs. 11.7 ± 4.2 ± 10^6 cells/ml; P < 0.001) and macrophages (2.5 ± 1.0 ± 10^6 vs. 9.2 ± 3.2 ± 10^6 cells/ml; P < 0.01). MMP-2 and MMP-9 activities were determined by gelatin zymography.

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 inflammatory effects of Cd by reducing lung parenchyma inflammatory cell infiltration.

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The effect of abnormal savda münzq on morphological and ultrastructural changes of articular cartilage in an abnormal savda syndrome animal model

Results: Microscopic examination of the synovial membrane showed swelling of hyaline articular cells, interstitial angioectasis in the hypothalamus; there were moderate and severe pituitary corticosterone hyperplasia, moderate and severe adrenal cortex and medulla hyperplasia, especially zona reticularis cell of adrenal cortex which maintained its normal architecture. In the hypothalamus of the drug intervention groups, the morphological and ultra-structural changes were diminished in a dose-dependent manner.

Conclusion: This animal model reproduces abnormal Savda syndrome of traditional Vythgor medicine causes anatomical changes in the HPAA axis, that are opposed in a dose-dependent manner byASM. 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 in a rat model

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 to:

1. Assess in vivo age-related cartilage changes and to follow osteoarthritis (OA) in rat knees from three dimensional (3D) datasets by 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 MR at 3 months of age, 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 contralateral). Cats were imaged at 3 months, 6 months and 9 months after surgery.

Results: High-resolution (51 x 51 x 94 μm voxel size) 3D Gradient Echo with fat suppression sequences were performed with a specific home-made 2- elements matrix. Animals were trained to be MR friendly. Histological examination of 5 μm sections (5 μm, Haematoxylin-Eosin-Safran, Toluidine Blue, Sirius Red), corresponding to selected MRI weight-bearing areas, were achieved. For the two imaging modalities (in vivo and ex vivo), cartilage volume divergences were observed on a slice-by-slice basis. The resulting masks were then used to compute the knee cartilage volume and thickness (histology-MRI confrontations).

Conclusion: The 3T MRI at 7T allows in vivo analysis of three different age groups of rat knee cartilage. Quantitative 1.5T-MRI values are compatible with expected thickness changes as depicted histologically. A significant correlation between the two methods is established. 7T 30 MR-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-τ in human macrophages: involvement of cellular antiviral factors and IL-6

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 adhesions and macrophages were obtained after 7 days of differentiation. Human macrophages are chronically infected by HIV.

Results: IFN-τ efficiently inhibited the early steps of HIV biological cycle, decreasing intracellular HIV RNA and inhibiting the initiation of the reverse transcriptase of viral RNA to protein DNA. Two major mechanisms of 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 or (ii) IFN-τ via JAK/STAT-6, which are natural ligands of CRCC, the main co receptor of HIV on macrophages. These results suggested that IFN-τ induces the same antiviral pathways in macrophages as the type I IFN are, 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-1β or TNF-α, in contrast with IFN-β/L-6-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, IFN-β 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-τ.
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Involvement of ASICs in colonic hypersensitivity induced by butyrate in rats

| Matricon a, A Gelot b, N Volety b, L Madhu nck c, S Bourdu a, A Escalier a, D Arditi c, "Clermont-Ferrand – France; a Montpellier – France; b Nimes – France; c Clermont – France. Abstract 268 |

Introduction: The treatment of irritable bowel syndrome (IBS) characterized by colonic hypersensitivity, abdominal pain and bloating, is empirical and often poorly based on the fact that butyrate can help with colonic hypersensitivity. Consequently, the involvement of acid sensitive ionic channels (ASICs) was investigated in the model of colonic hypersensitivity.

Methods: An in vivo study was performed in a 200 rats, treated with any of 3 days and colonic hypersensitivity was evaluated at the end of intracolonic instillations (24 hours). Firstly, cPTX141 (40 mg and 10 mg) or an 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 RCR-PCR test) or chronic (butyrate treatment) manner at a dose of 1/2000. CTX141 was performed in DRG in order to localize their nociceptive fiber expression.

Results: Butyrate enemas induced a colonic hypersensitivity with a colorectal reaction threshold of 4.3 ± 0.7 mg/mL at the end of intracolonic instillations. Control rats treated with saline by intrarctal route during 3 days had a colorectal reaction threshold around 60 mg/mL. CTX141 at the dose of 40 mg and 80 mg significantly reversed the decrease of colorectal threshold in butyrate-treated rats (59.5 ± 5.6 and 62.7 ± 4 respectively vs. vehicle score, P < 0.05). Secondly, in the acute vehicle, ASIC1A mRNA expression was <10% of the vehicle), whereas calcium chloride (1 mM) (1 μg/g, 1 μmol/g, and 80 μmol/g, respectively) increased ASIC1A mRNA expression by 185%, 250% and 800% respectively vs. vehicle score, P < 0.001). A chronic treatment with metformin (200 mg/kg/day for 2 weeks) induced a significant decrease in hyperglycaemia (P < 0.05), in hyperglycaemia (P < 0.01) and clearly improved platelet aggregation (P < 0.05). Metformin also stabilized the expression of hyper-glucosidase.

Conclusion: In conclusion, metformin-fed rats with this moderately high fat diet may be a model of interest for the in vivo testing of novel antiadipogenic compounds.

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Effect of Ceiba pentandra extract on streptozotocin-induced type-1 diabetes in rats

| P Dureuil-Djoum a, L Télong b, MC Tchamadeu c, T Dimo c, P Kametchoug a |

Introduction: An animal model mimicking the evolution of human type 2 diabetes in rats was used for evaluating novel pharmacological interventions. The study concluded that C. pentandra possesses antiadipogenic 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 on immune-tolerance

| P2T Abstracts 2007 |

R Cacho a, P2T Abstracts 2007; 1 – 99

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SR59119A, an ADRB3 agonist, reverses myometrial TNF-alpha-dependent apoptosis, and cytokine over-expression in a LPS inhuman in vitro model of preterm labour/infusion

| F Lirussi a, R Zakotoniaina a, S Madani a, P Goirand b, M Breuillier-Fouché b, M Leroy a, M Dumont a, N Tricot c, P Saito a, M Baudou Dijon – France; a Montpellier – France; b Fontenay aux Roses - France |

Introduction: The immunosuppressive pathway mediated by the kynurenine pathway (KP) plays a major role in the immunobiology of tolerance. In this context, regulatory T cells (Treg) play a key role. The expression of indoleamine-2,3-dioxygenase (IDO), an enzyme involved in the kynurenine pathway, is up-regulated in the placenta during pregnancy. IDO is thought to play a role in the suppression of maternal immune response. IDO inhibition is considered as a potential therapeutic approach to prevent preterm labour. The study concluded that SR59119A, an ADRB3 agonist, reverses myometrial TNF-alpha-dependent apoptosis, and cytokine over-expression in a LPS inhuman in vitro model of preterm labour/infusion.
Methods: Myometriu biopsies were obtained from pregnant women who delivered by caesarean section. Biopsies were placed in a 24 well plate and leased to stabilize at 37°C for 48 h, then stimulated with LPS 10 μg/mL in the presence, or not, of SARM9119A (10−4 to 10−1 M) or TNF-alpha antibody (TNF-alpha-α, 0.6 ng/mL) in order to characterize 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 by flow-cytometry analysis.

Results: Compared with controls, LPS stimulation was associated with a significant increase of cleaved caspase-3 protein (in ADU 94.1 ± 134, 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, the luciferase activity. Indeed, the AM promoter luciferase reporter gene assay in 3T3-F442A cells, we have shown that the AM response to insulin is mediated by IRIE.

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.

376 Saponin effects on apoptosis in human rheumatoid arthritis synoviocytes. Relationship with cyclooxygenase-2

B Liagre, P Vergne-Salle, DY LeGER, JL Charissoux, R Treves, P Berzin, JL Benettel

Introduction: In conjunction with rheumatoid arthritis Therapeutic ‘Limoges - France’

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 Rho phosphorylation. We observed a significant increase of initially low Rho activities between the 1 and 3 mM hecogenin or tigogenin concentrations. Between passages 4 and 8, after 48 h of culture, Rho was 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-1, 8 and 9 (R&D Systems) and protein level expression of cleaved caspase-3. Cytokines production was assessed by flow-cytometry analysis.

Conclusion: This study suggests that inflammation triggers, through a TNF-α-dependent pathway, myometrium apoptosis that is partially reversed by the anti-inflammatory drugs. In our conditions LPS was associated with a significant increase of IL-4, IL-5 and IL-6 and IL-8 at 48 h. SARM9119A strongly reduced the over-expression of IL-6 and IL-8 (in pg/mg, IL-8: 16482 vs. 5250; IL-6: 61861 vs. 31701 for LPS and LPS + SARM9119A 10−4 M respectively).

Disclosure: this work was supported by La Société Française de Rhumatologie.

377 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 possess both antiresorptive and anabolic effects. It has been previously shown that strontium ranelate stimulates murine osteoblastic differentiation while decreasing their osteoclastogenic abilities.

Methods: Primary mouse calvaria cells were treated with strontium ranelate (0.1, 0.3 and 1 mM of Sr2+) 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 nodules.

Results: Whatever the strontium ranelate treatment period during the culture, osteoblasts expressed higher mRNA level of osteoblast markers (alkaline phosphatase, osteocalcin) and protein level expression (determined by real time polymerase chain reaction) and alizarin red staining for bone formation and decreasing bone resorption. The present study assessed strontium ranelate effects on osteoblast differentiation and their abilities to induce osteoclast differentiation.

Conclusion: These findings show that strontium ranelate has an anabolic effect on bone formation through osteoblastic differentiation together with a decrease in osteoclast differentiation due to the dual mechanism of action of strontium ranelate on bone formation and resorption.

378 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 phosphate activity, mineralization, and prostaglandin E2 production in mouse osteoblasts.

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 osteoblast differentiation in vitro. We used murine marrow stromal cells from mice knockout for cyclooxygenase-2, the enzyme responsible for the prostaglandin E2 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 bone formation.

Results: After 14 days of culture, 1 and 3 mM strontium ranelate increased alkaline phosphatase mRNA expression by 2.0-fold (P < 0.01 vs. control) and 3.0-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% compared with wild marrow stromal cells from wild type mice, and there was no increase in osteocalcin mRNA expression with either concentration of strontium ranelate. After 21 days of culture, 3 mM strontium ranelate increased osteocalcin mRNA expression (P < 0.01 vs. control) in murine marrow stromal cells. Wild-type mice 3-fold relative to controls. The strontium ranelate-induced increase in osteocalcin expression was abrogated in
Effects of formoterol on repeated cadmium inhalation-induced lung inflammation and emphysema in rats

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Introduction: The aim of the present study was to analyse the possible involvement of LPA in the development of renal 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 gluc- and lipotoxicity

Y. Van Boeckel, D. Multon, B. Courtois, V. Gustin, M. Montpellier - France

Introduction: Pancreatic beta cell dysfunction and insulin resistance are the hallmark of type 2 diabetes. Under diabetic conditions, an increased flux of glucose and fatty acids (respectively named as gluco- and lipotoxicity) 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/ASK), NF-kappaB, and nuclear factor-kB), 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 lipotoxicity.

Methods: INS-1 insulin secreting cells were incubated in the presence of various concentrations of H2O2 and a mixture of glucose and oleic acid (glyco-lipo-toxicity). Effects of an antioxidant (N-acetyl cysteine, NAC) and of two specific inhibitors of p38MAPK (SB203580) and JNK (SP600125) were also determined on these insulin effects.

Conclusion: p38MAPK and JNK phosphorylation were detected as soon as 5 min after initiating incubation of cells in the presence of H2O2. Concentration-response curve obtained was biphasic, with a maximum level of phosphorylation occurring for 10 nM H2O2. Total protein expression did not change. Insulin secretion induced by 8.3 mM glucose was inhibited when concentrations of H2O2 increased. Inhibition was detectable for 0.01 mM and maximal for 0.1 mM H2O2. Concentrations higher than 1 mM H2O2 were lethal to the cells. The SB203580 and SP600125 partially prevent the impairment of cell functionality in the presence of 0.05 mM H2O2. Their effects are not additive.

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 glyco- and lipotoxicity and thus to define a possible relationship between stress-sensitive pathway activation and impairment of insulin secretion.

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Renal effects of meloxican, a COX-2 preferential inhibitor, in comparison with a non selective COX inhibitor, ketoprofen, in anesthetized pigs

S. Junot1, B. Trocny2, S. Kerou1, J.M. Bonnet-Garin1, J-P. École1, S. Keroak1, J.G. Liege - BELGIQUE; J. P. Baulant - FRANCE

Introduction: COX-2 selective NSAIDs have been largely covered, but conflicting data exist on renal effect of meloxicam, a COX-2 preferential NSAID. The effects of an antinociceptive (N-acetyl cysteine, NAC) and of two specific inhibitors of p38MAPK (SB203580) and JNK (SP600125) were also determined on these insulin effects.

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384 Reversible down-regulation of MMP-9 and MMP-12 gene expression in human primary macrophages treated by arsenic trioxide

E Bourdonnay\textsuperscript{a}, C Morazec\textsuperscript{a}, C Martin-Choulhy\textsuperscript{b}, I Vermhet \textsuperscript{a} \textsuperscript{a}Laboratoire de Pathologie, UMR 7507, INSERM U7507, 75015 Paris - France; \textsuperscript{b}Laboratoire d'Immunologie, UMR 7558, INSERM U7558, 75015 Paris - France

Introduction: Chronic exposure to inorganic arsenic, a carcinogenic environmental contaminant present in cigarette smoke, is associated with immunosuppression. We recently showed that, in vitro, non cytotoxic arsenic trioxide (As2O3) markedly impairs endocytosis and phagocytosis activities of human primary macrophages, some major immune cells. In addition, As2O3-treated macrophages displayed 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 U/ml GM-CSF, which strongly increases the expression of both MMP-9 and MMP-12 gene expressions after a 6-day culture. Macrophages were then treated with non cytotoxic concentrations of As2O3 (0.1–1 \mu M) 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 expression was almost fully reversed when As2O3-treated macrophages were next cultured in arsenic-free medium.

Conclusion: Our results 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 can ‘de-differentiate’ human macrophages into monocytic-like cells.

385 Valvular heart regurgitation and pergolide: a French observational study

M Marin\textsuperscript{a}, J Eloit\textsuperscript{b}, P Ladure\textsuperscript{a}, E Foveau\textsuperscript{a}, B Lebrun-Vignes\textsuperscript{a}, B Lebrun\textsuperscript{a} \textsuperscript{a}Hôpital de la Pitie\textsuperscript{-}Salp\text{é}tr\text{é}re hospital and a meta-analysis of similar trials. To investigate this issue we conducted an observational study at the Pitie\textsuperscript{-}Salp\text{é}tr\text{é}re hospital and a meta-analysis of similar trials.

Introduction: Valvular heart regurgitation is frequently associated with pergolide use. We recently demonstrated 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 displayed 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.

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added for giving a brilliant and more attractive aspect. The potential medical consequences of these activities include increased heart disease and strokes. It is therefore crucial that the observed peer seems too big to go directly in the low respiratory tract.

388 Fenofibrate, a PPAR alpha agonist, exerts neurological recovery-promoting, anti-inflammatory and anti-oxidative effects in traumatic brain injury NR Chen*, U. Besson*, B Palmiter*, Y Garcia*, M Plotkine*, C Marchand-Leroux* *Pars - France

Introduction: We previously demonstrated that fenofibrate, a peroxisome proliferator-activated receptor α (PPARα) agonist, reduced the neurotoxic cell death and 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 a further elucidation of the potential therapeutic effects of fenofibrate, 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 trauma. A neurological assessment was done 24 h after TBI score ranging from 0 (no sign of trauma) to 9 (total brain death). Then rats were killed and the brain MMP9 expression, total glutathione (GSX), oxidized glutathione (GSGx) levels were determined. The same staining 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 ± 0.8 vs 10, P < 0.05) and suppressed tissue injury, COX2 expression (P < 0.05), MMP9 (P < 0.001), decreased the 3NT, 4HNE staining. Our data suggest that PPARα activation could mediate pleiotropic effects and strengthen that it could be a beneficial property on reducing inflammation and enhancing endothelial dependent vasodilation in cephalic vessels. The purpose of this study was to assess the effects of simvastatin pre-treatment on cardiopulmonary bypass induced cerebral vascular injuries.

Methods: Male Sprague Dawley rats were randomly allocated to the simvastatin pre-treated group (10 mg/kg, 14 days) or a non-treated group. Animals underwent a 30 minutes cardiopulmonary bypass procedure or sham surgery. Animals were sacrificed at 24 h post-operation. TUNEL assay was performed 24 hours after the procedure (T24). In vitro middle cerebral artery reactivity, systemic inflammation evaluation by measuring plasma concentrations of TNFα and immunochemistry staining of ICAM-1 and the high mobility group box 1 antibody and a NeuN 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 cerebral-vascular injuries of cardiopulmonary bypass. This treatment could be a therapeutic option to non-cognitive impairments following cardiopulmonary bypass.

389 Agomelatine efficacy on major sleep disturbances in Smith-Magenis syndrome: an exploratory, open study in children H de Leersnyder*, A Fabián*, C de Boilhat* *Paris – France; **Ceurbevoie - France

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 (SD) with sleep fragmentation, wake after sleep onset and early awakening are frequent in SMS. Agomelatine is a new melatonin-receptor agonist and a serotonin-2C receptor antagonist that showed good efficacy in the treatment of sleep disturbances in SMS. The aim of this study was to assess the potential efficacy of agomelatine in the 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). Acetobutol (10 mg/kg o.d. in the morning) (β1 antagonist) was co-administered in order to block endogenous 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 the sleep diary and the children’s sleep questionnaire, which showed that the nocturnal wakening 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 acetobutol (10 mg/kg), was an effective and well-tolerated treatment of sleep disturbances in SMS. On the family requests, eight patients are now at present still treated with this combination since 4 years 1 month and will be followed up to 2002.

Additional trials are needed to confirm the therapeutic potential of agomelatine in SMS.
Activation of nucleus tractus solitarius 5-HT₃, but not other 5-HT₂ receptor subtypes inhibits the sympathetic activity in rats

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*Paris – France

Introduction

In a previous study, we showed that nucleus tractus solitarius (NTS) 5-HT₃ receptor stimulation elicits a sympathetic excitation, without affecting heart rate. Conversely, the activation of NTS 5-HT₄ receptors produces the typical responses of baroreceptor activation: hypotension and bradycardia. However, to date, the receptor subtype underlying the cardiovascular responses to 5-HT₄ receptor agonists, produced a decrease in blood pressure and in heart rate. The maximal cardiovascular changes evoked by DOH (0.5 pmol) could be almost completely abolished by prior intra-NTS microinjection (10 pmol) of MDL-100907, a selective 5-HT₄ receptor antagonist, but not by 5-HT₁ or 5-HT₂ 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-HT₄ receptor stimulation. In an ongoing investigation, we assessed the effects of NTS administration of a subthreshold (0.05 pmol) dose of DOH, known to facilitate the baroreflex, on the vascular component of the baroreflex, which might also modulate the sympatho-hydraulic reflex. We then investigated whether intra-NTS microinjections of selective agonists of 5-HT₃ receptor subtypes, on BP and HR baselines.

**Methods:**

In order to address this question, we first analysed, in pentobarbital-anesthetised rats, the effects of intra-NTS microinjections of selective agonists of the different 5-HT₃ receptor subtypes, on BP and HR baselines.

**Results:**

Under these conditions, 2-5-dimethoxy-4-iodoamphetamine (DOI), a wide spectrum 5-HT₂ receptor agonist, but not selective 5-HT₃A and 5-HT₃C receptor antagonists, 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-HT₃C receptor antagonist, but not by 5-HT₁ or 5-HT₂ 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-HT₄ receptor stimulation. In an ongoing investigation, we assessed the effects of NTS administration of a subthreshold (0.05 pmol) dose of DOH, known to facilitate the baroreflex, on the vascular component of the baroreflex, which might also modulate the sympatho-hydraulic reflex. We then investigated whether intra-NTS microinjections of selective agonists of 5-HT₃ receptor subtypes, on BP and HR baselines.

**Conclusions:**

The 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.

Consumption of benzodiazepines among drug addicts in Île de France area: course over 5 years

S. Djazairi, X. Micallef-Rollod, S. Dally

*Paris – France

Methodology

The centers of intervention for the use of illegal drugs and dependence (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 of interest.

**Methods:**

We propose to examine with OPPIDUM the evolution of the consumption of benzodiazepines and related (BZD) among the patients consulting in addictions structures of care specialized in Island of France between 2000 and 2008.

**Results:**

A total of 29% of outpatients reported to consume benzodiazepines in the last year (BZD). They are mostly males (72%) and their mean age is 37.4 years. 61% of them lived under unfavourable socio-economic conditions. A polycosumption of psycho-active substances is noted in 96% of the cases with an average number of products of 1.3. The BZD are consumed orally in 98%, in a daily way in 82% and as more than 1 pill in 35% of the cases. The most frequently used BZD is the flunitrazepam, the BZD 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 benzodiazepines lead to the substitution of BZD by other BZD such as zopiclone. This trend is confirmed by the Nots data (other data of spontaneous notification).

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 (MT₁, MT₂) receptors and 5-HT₂C (5-HT₃) receptors. Preclinical data and data from clinical studies, showed that nucleus tractus solitarius (NTS) 5-HT₃ receptor stimulation elicits a sympathetic excitation, without affecting heart rate. Conversely, the activation of NTS 5-HT₄ receptors produces the typical responses of baroreceptor activation: hypotension and bradycardia. However, to date, the receptor subtype underlying the cardiovascular responses to 5-HT₄ 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-HT₃C receptor antagonist, but not by 5-HT₁ or 5-HT₂ 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-HT₄ receptor stimulation. We then investigated whether intra-NTS microinjections of selective agonists of 5-HT₃ receptor subtypes, on BP and HR baselines.

**Methods:**

A total of 121 non-depressed patients with DSM-IV GAD were randomized to agomelatine (25-50 mg) 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 scales deep sleep evaluation questionnaires (SUDEQ), and the Sheehan Disability Scales (SDS).

**Results:**

The main analysis on the last HAM-A total score change from baseline demonstrated the efficacy of agomelatine compared to placebo [H (SE) = -3.28 (1.58); 95% CI = [-6.41; -0.15], P = 0.040, ANOVA]. 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.

Lactobacillus acidophilus modulates intestinal pain and inhibits opioid and cannabinoid receptors


**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 antibiotic (ceftriaxone) treated (ABX) Escherichia coli (E. coli) to induce expression of analgesic receptors (MOR and CR2 receptors) was evaluated on human IHT-29 epithelial cells. The functional role of L. acidophilus NCM-induced analgesic receptors was investigated by assessing the colonic perception of rats using a validated technique of colorectal distension.

**Results:**

L. acidophilus NCM induced a sustained increase of OPN/MH mRNA expression 1 hour after bacterial stimulation, the induction was of the same magnitude as that observed with the positive i.e. TNF-a. NCM strain was able to induce significant CNR2 mRNA expression compared to resting epithelial cells. Expression of opioid receptors (MOR and CR2) in IHT-29 epithelial cells incubated with the L. acidophilus NCM strain. In rodents, L. acidophilus NCM administration at a clinically relevant concentration of 10⁶ CFU / d during 15-constitutive days induced MOR1 and CR2 protein expression in 25-60% of epithelial cells. In untreated rats, a mean colorectal distension of 50 ± 2 mmflg 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 NCM strain (10⁶ 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 the colonic hypotension evoked by the L. acidophilus NCM strain was abolished by 44% by the colorectal distension threshold compared to untreated animals, exerted an anti-inflammatory effect at the same magnitude as 1 mg/kg of morphine, and we observed a reduction of the nociceptive pain response of morphine used at 0.1 mg/kg. L. acidophilus NCM-induced analgesia was significantly inhibited by peritoneal administration of the CR2-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.
Inhibition of cardiac reflex responses by direct stimulation of periaque- 
 turbulent -2-one. Further pharmacological studies are required to explain 
 the relationship in the hypnotic and anticonvulsant activities of the novel 1, 5-
 benzodiazepines: the 4- (2-hydroxyphenyl) -1, 5 benzodiazepine-2-one 
 respectively compared to those of flunitrazepam and diazepam as drug references.

Methods: The two novel 1, 5-benzodiazepines and the drug references 
 against the clonic, tonic seizures and mortality (P< 0.001).

Conclusion: The present study showed the importance of the activity 
 in mice. A total of 250 mice Swiss albino (17 male and 79 female), 10–12 weeks old, has been used. The hypnotic 
 in the hypnotic and anticonvulsant activities of the novel 1, 5-
 benzodiazepine-2-one. The two activities were compared to those of flunitrazepam and diazepam as drug references. 

Methods: Twelve young male volunteers completed this double-blind, placebo and methylphenidate controlled, randomized, cross-over study, after informed consent. Bupropion 
 and peripheral effects of a 2-week bupropion administration: 

Methods: Twelve young male volunteers completed this double-blind, placebo and methylphenidate controlled, randomized, 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 

Behavioural and peripheral effects of a 2-week bupropion administration: 

TRAAK, a potassium channel involved in polymodal pain perception

Methods: All experiments were performed on 20–24 g male C57Bl/6 J mice. TRAAK knock-out (TREK1/2 and TRAAK double knock-out mice of the N10 F2 
 backcross generation to C57Bl/6 J congenic strain. All mice were acclimated to 

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Pharmacological screening of two novel 1. 5-benzodiazepine-2-ones. 

A total of 250 mice Swiss albino (17 male and 79 female), 10–12 weeks old, has been used. The hypnotic 
 the laboratory conditions for at least 1 week prior to testing. They were housed in groups of five in a temperature-controlled room. The lights were on during the light phase of the natural photoperiod and off during the dark phase. The mice were fed ad libitum. The behavioral experiments (thermal, mechanical and chemical pain tests 

Methods: Twelve young male volunteers completed this double-blind, placebo and methylphenidate controlled, randomized, 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 

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Levodopa effect on motor activity in Parkinsonism: a PET study

Conclusion: 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 activity 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.

Conclusion: Altogether, these results suggest that, in stressful situations, activation of the cuneiform nucleus may produce dAPG neuroexcitation, which causes (i) B3 activation at the origin of 5-HT release within the NTS, (ii) local 5-HT 

Methods: Methods: Two hundred and twenty volunteers completed this double-blind, placebo and methylphenidate controlled, randomized, 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 

Conclusion: The Bezold-Jarisch bradycardia was strongly inhibited (–80%) during dAPG electrical stimulation. Intra-NTS microinjections of granisetron (175 pmol), a 5-HT3 receptor antagonist, as well as D,L-norfenfluramine (175 pmol), a 5-HT1B receptor agonist, prevented the effect of dAPG stimulation. Muscimol microinjection into B3 also prevented dAPG-mediated inhibition in contrast, the cardiac reflex response was inhibited (~75%) following B3 activation by DE-Homocystic acid (30 mmol), and the effect of B3 activation could be dose-dependently prevented by intra-NTS microinjections of granisetron (175–250 pmol). Using anterograde (Phaseolus vulgaris leucoagglu-

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Behavioural and peripheral effects of a 2-week bupropion administration: a placebo and methylphenidate controlled, randomized, double-blind 

Introduction: Ion channels play an 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 or 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 are activated by hypothermia. They would contribute to the temperature 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-1/2 knock-out mice to evaluate the role of these K+ channels in pain perception associated with different types of stimuli.

Methods: All experiments were performed on 20–24 g male C57Bl/6 J mice. TRAAK knockout (TREK1/2 and TRAAK double knock-out mice of the N10 F2 

Conclusion: The present study showed the importance of the structure-activity relationship in the hypnotic and anticonvulsant activities of the novel 1. 5-benzodiazepine-2-one. Further pharmacological studies are required to explain the role of the pharmacophores in the mechanisms of benzodiazepines activities.

Conclusion: Together, these results suggest that, in stressful situations, activation of the cuneiform nucleus may produce dAPG neuroexcitation, which causes (i) B3 activation at the origin of 5-HT release within the NTS, (ii) local 5-HT receptor stimulation and (iii) consequent inhibition, via GABAa and NK; receptor activation, of cardiac reflex bradycardia.

Conclusion: Like TREK-1, TRAAK appears as an important ion channel for 

Conclusion: The present study showed the importance of the structure-activity relationship in the hypnotic and anticonvulsant activities of the novel 1. 5-benzodiazepine-2-one. Further pharmacological studies are required to explain the role of the pharmacophores in the mechanisms of benzodiazepines activities.

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Conclusion: The present study showed the importance of the structure-activity relationship in the hypnotic and anticonvulsant activities of the novel 1. 5-benzodiazepine-2-one. Further pharmacological studies are required to explain the role of the pharmacophores in the mechanisms of benzodiazepines activities.
404 Is hypocretin involved in stress-induced sleep alterations in mice? A Rachalski†, J Adrien†, M Hannon†, V Fabre* *Parijs – France

Introduction: Hypocretin, also known as hypocretin, is known to activate cerebral structures involved in sleep regulation such as Raphe nuclei (R). Conversely, R serotoninergic neurons inhibit the hypocretin system. Hcrt and serotonin are thus key neuromodulators of sleep/wake cycle which both exert a effect on REM sleep. Interestingly, compared with wild-type (WT) mice, serotonin transporter deficient (5-HTT−/−) mice exhibit modulations of sleep regulation induced by REM sleep deprivation and absence of REM sleep after restraint stress. The aim of our study was to specify interactions between hypocretinergic and serotoninergic 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-proh hypocretin 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, polysomnography was used to evaluate mice response to immobilization stress and the effects of specific hypocretinergic receptor 1 (hcrtr1) blockade by SB-334867 (3 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 hcrtr1 levels in RN and pre-proh hypocretin mRNA levels in the lateral hypothalamus). Under basal conditions, 5-HTT−/− mice exhibited higher tissue levels of hcrtr1 in RN and changes in pre-proh hypocretin levels. Immobilization stress also activated hypocretinergic neurons in mutants and increased hcrtr1 levels in RN. In absence of stress, acute hcrtr1 blockade by SB-334867 did 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 in naive WT mice.

Conclusion: Altogether, we showed an alteration of hypocretinergic neurotransmission in 5-HTT−/− mice and a stimulatory effect of stress on hypocretinergic neurons (both at basal conditions and paired with immobilization stress). Our data support the existence of functional interactions exist between hypocretinergic and serotoninergic systems under basal conditions and after immobilization stress. Accordingly, the hypocretin system appeared to play a role in stress-induced sleep alterations.

405 Both risperdone and clozapine but not haloperidol nor chlorpromazine increase focal cerebral ischemia severity in rat J. Deplanque†, D. Ferrand†, J. Adrien† and M Bourin* *Clermont–France

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). The cross-sectional Doppler study was conducted in 2005 in the Midi Pyrenees Region to describe the prevalence and treatment of chronic pain in PD patients.

Methods: Totally 450 patients 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 [PFDI 99]) and analgesic consumption. Statistical analysis was performed using the R software (version 2.11). Results: Chronic pain was twice more frequent in PD patients than in control patients (47.9 vs. 19.4% [P<0.05]). The 3 groups were not matched for 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 (IVAS = 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 context. 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 disorder, had 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 as an heterogeneous disease group. This study confirms that the prescribed analgesics has ever been appropriately assessed in PD patients. This needs to be further explored.

406 Behavioural assessment of two new drugs (pregabalin and duloxetine) potentially effective in neuropathic pain treatment B Ling†, F Coudreou†, A Eschalier†, N Authier* *Clermont Ferrand – France; Clermont-Ferrand – France

Introduction: We developed two animal models of nociceptive sensory neurology pathologically induced by repeated or acute administration of oxaliplatin in which treated animals reproduce the characteristic features of neuropathic pain observed 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 hyperalgesic neuropathic pain model, we used 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. Behavioral sign assessed to assses nociceptive threshold was cold thermal allodinia, 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 (p = 0.001 and 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 100 mg/kg) did not produce any effect. Under the antidepressant anti-nociceptive effect at 105 min (+38%, 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): magnesium > venlafaxine > duloxetine > gabapentin > carbamazepine × pregabalin for the acute model and magnesium = venlafaxine > duloxetine > gabapentin 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 may be used in clinical trials. Pregabalin seems to be more effective in chronic than acute model, suggesting different neurotoxic mechanisms between these two models.

407 Effect of benzodiazepine ligands on the anxiolytic-like activity of antidepressants in the four-plate test in mice R V. Dabouis, N Cogheau and M Bourin* *Nantes – France

Introduction: Benzodiazepines (BZDs) remain the first choice drugs for the treatment of anxiety disorders but these compounds involve several side effects (lack of efficacy, physical addiction, sedation and so on). Recently, two selective serotonin reuptake inhibitors (SSRIs) have proved their efficacy in the treatment of anxiety disorders in humans. Preclinical studies have demonstrated that the SSRIs and SNRIs potentiate the anxiolytic effect of DOI (5-hydroxyindole-3-acetic acid, DOI) in the 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 animal crossing. The anxiolytic-like effect of these antidepressants is facilitated by the serotonin 1A receptor (5-HT1A) receptor agonist). The aim of the present study was to determine the potential role of the specific serotonin reuptake inhibitors (SSRIs) and of benzodiazepine ligands co-administration in the FPT. We have co-administered two selective serotonin reuptake inhibitors, paroxetine and citalopram; two benzodiazepine ligands co-administered (lorazepam, alprazolam and diazepam) and benzodiazepine receptor antagonists (flumazenil) on the FPT.
Methods: In the first time, the sub-active doses of antidepressants (ADs) were co-administered with active doses of benzodiazepine receptor antagonists. In the second time, the active doses of ADs were co-administered with inactive doses of benzodiazepine receptor antagonist in the PPT. 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 (0.1 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 (6 mg/kg), when only flumazenil (8 mg/kg) inhibited the anxiolytic-like effect of flumazenil (8 mg/kg). Only, flumazenil (8 mg/kg) decreased anti-apoptosis and antiallodynic effects 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 neuroprotective system, their anxiolytic-like effects in the PPT in mice are differently influenced by benzodiazepine ligands.

410 Comparison of the effects of the 5-HT3 receptor antagonists on pain and anxiety in rodents

Introduction: The potential memory-enhancing properties of two dopaminergic 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: Piribedil (10 mg/kg) and Bromocriptine equally enhanced performance in the object recognition test. Young adult rats (experiment A), only Piribedil displayed beneficial effects against age-related memory impairments in two radial maze experiments in mice. (Experiment B), a two-stage paradigm of spatial discrimination was used. Piribedil was used at 1 and 10 mg/kg s.c. 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 rats were used, 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 were slightly improved as compared to aged animals’ level.

Conclusion: The restoration of specific mnemonic impairments in mice highlights the potential interest of Piribedil in treating cognitive symptoms of Parkinson disease.

411 Consumption of cannabis among subjects with history of abuse/independence or under an opiate maintenance therapy: OPPIDUM data in 2004 and main trends since 2000

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) is a nationwide 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 year.

Results: During October 2004, 3373 subjects were included. 41% (n = 1391) 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 frequent for the sub-group of subjects with a lower motivation to stopping (38% of suffering when stop cannabis, 65% of daily consumption, 37% of alcohol concomitance, 71% of description of abuse or dependence with cannabis).

Conclusion: This study underlines the importance of a counseling at the exclusive cannabis use in the drug dependent patients recruited by specialized care centres. This sub-group has 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 light against illicit drug, tobacco, alcohol 2004–2008.

412 Triggering factors for one-trial tolerance in the four-plate test retest in mice

Methods: Adult male Sprague-Dawley rats underwent chronic unilateral injury (i.e. Loose ligatures) of the sciatic (extracerebral) or infrarobital (cerebral) model nerve. Mechanical hyperalgesia and allostynia were measured using Ugo Basile analgesiometer and von Frey filaments, respectively. Nociceptive reaction thresholds were markedly reduced 14 days after ligation of the sciatic nerve (paw withdrawal threshold). 71% of animals were of great pain (4.5±0.5 g) by 16 ± 4 g, 18 ± 3.7 g and 21.8 ± 3.8 g (pre-operatively, respectively) and the infrarobital nerve (0.34 ± 0.05 g v.s. ≥ 12.0 g, p<0.05). At that time, CGRP- or 5-HT7-receptor ligands were administered, and results were expressed as percentage of the response measured at regular time intervals.

Results: Acute 5-HT7-receptor blocker (SB-269970, 3 mg/kg, i.p.) partially reversed hyperalgesia in rats with sciatic nerve ligation (only on vasculation, 45-75 min after administration), but was ineffective in rats with infrarobital nerve ligation. In contrast, 5-HT7-receptor stimulation (AS-19, 10 mg/kg.s.c.) exerted a long-lasting and strong 15±1 h, infrarobital nerve ligation; more than 18 hours, sciatic nerve ligation) of great pain (4.5±0.5 g; 18 ± 2.5 g and 21.0 ± 3.8 g, respectively) and the sciatic nerve ligation (4.5±0.5 g v.s. ≥ 12.0 g, pre-operatively). At that time, CGRP- or 5-HT7-receptor ligands were administered, and results were expressed as percentage of the response measured at regular time intervals.

Conclusion: This study underlines the importance of a counseling at the exclusive cannabis use in the drug dependent patients recruited by specialized care centres. This sub-group has 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 light against illicit drug, tobacco, alcohol 2004–2008.
Conclusion: The loss of effect observed with diazepam during trial 2 with four-plate test may also as a one-trial transient 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 only cause of this loss of drug effect which is observed only as a potentializer; 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 model to study the effects of diazepam in a test of anxiety and to dissect mechanisms of action of this compounds: because the presence of two opposite drives creates a situation of risk assessment deeply implied in anxiety. Furthermore, punishments represent an instrument to sensitize the results obtained in four-plate test.

413 Clinics and pre-clinics studies of the endogenous and exogenous concentrations of GHB in brain tissues, its biological variations and use

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Introduction: Neurontin and neuromodulator of the GABA-ergic system, concentration of endogenous gamma-hydroxy-butyrate (GHB) was increase after death. In vivo, GABA is converted to GHB by the action of sucyclic semialdehydes rectasate, and from 1.4 Butanol and 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 death 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 determine 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 biomarker matrix should be used to identify voluntary or involuntary administration of GHB in different forensics expertises.

Conclusion: The biological interpretation of GHB concentrations was very difficult to realize: especially when samples were taking away out remotely of administration.

414 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 antimicrobial 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 may be 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 brain injury in different biological matrices.

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 (3.6 ± 1.15 pg/mg protein vs. 26.7 ± 3.08 pg/mg protein, P < 0.001). 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 < 0.04).

Conclusion: This study provides the first evidence showing the anti-edematous and anti-inflammatory effects of minocycline in a model of diffuse brain injury. The mechanism of action in our model is independent of p38 MAPK activation. Finally, our data provide a rational to test minocycline at least as an anti-edematous strategy, in severe head injury patients.

415 MRI investigation of brain lesions after prenatal hypoxia or hypoxia-ischemia

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Introduction: Deep and remarkable improvements in neonatology 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 nosy intra-uterine environment such as hypoxia or hypoxia-ischemia occurring during embryogenesis alters the neural development and provokes the appearance of a number of neural and neurobehavioral 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 its consequences (atrophy, necrosis, or haemorrhage) and to assess in vivo, non-invasively, the spatial distribution, extension and evolution of lesions.

Methods: Hypoxia-ischemia was reproduced 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 period. Fast T2-weighted, 1.5 T MRI (obtained by an Inspec C 1T30) 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. While matter thicknesses of adjacent brain regions were significantly different for the 2 groups, 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 in the brain parenchyma of severe hypoxic-ischemic or hypoxic animals. No such features were seen in controls. Highly severe multilocal necrotic lesions coupled with haemorrhage were found in the cerebrum of a number of pups, bearing an ominous 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 vitro 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). Moreover, we measured a white matter hypointensity, located necrotic and/or haemorrhagic lesions and assessed their evolution.

416 Acute neuroprotective effect of PPARY activator in a cerebral ischemia-reperfusion model: leucocyte/vessel interaction

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Introduction: In the course of cerebral ischaemia peroxisome proliferator-activated receptor-α activator induces protective neuroprotective effect in particular in the prevention of ischemia-induced leucocyte adhesion to cerebral vessels. The aim of this study is to bring to the fore in a cerebral ischemia-reperfusion model: (i) the neuroprotective effect of PPARY-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 PPARY-α activator, was administered by gavage twice a day during, 72 hours after onset of reperfusion. During this treatment period cerebral microcirculation was studied using leucocyte activation and vascular reactivity analysis thanks to intravital microscopy. The infarct volume was determined at 72 hours by histomorphometry.

Results: PPARY-α activation induced a significant decrease in infarct volume (23.4 ± 4.8 mm³) in comparison to vehicle-treated animals (40.0 ± 3.6 mm³, P < 0.01). In parallel fenofibrate treatment was associated to post-ischemic leucocyte protein adhesion expression in cerebral ischemia-reperfusion model. These results suggest the couple leucocyte-endothelium could be a PPAR-α activator, a target which could contribute to its beneficial effect in the course of cerebral ischemia.

417 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) enzyme is a critical mediator of cell death, and that its activator, matrix metalloproteinases-9 in cerebral ischemia (2). Matrix metalloproteinases-9 is known to degrade the basal membrane components of blood vessels and to contribute to the process of reperfusion injury. We have previously evaluated the effect of PJ34 (N-(6-oxo-5,6-dihydro-phenanthridin-2-yl)-N-N-dimethylacetamide), a PARP inhibitor, on intracerebral haemorrhage subsequent to a focal 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), xylazine hydrochloride (20 mg/kg). Animals received all the surgery except the artery occlusion. PJ34 (0.25 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 higher the score, the higher the deficit). Animals were then killed and cerebral tissue removed for the evaluation of intracerebral haemorrhage and infarct volume. Intracerebral haemorrhage, defined as either visible or histological, was assessed in a series of horizontal and coronal brain slices every 500 μm interval.

Results: Saline-treated ischemic mice showed a significant increase in the score of ischemia haemorrhage and infarct volume, compared to sham-operated mice (5 ± 2 and 4 ± 3 respectively) 48 h after the onset of
Methods: Arterial pressure and renal nerve activity were simultaneously recorded in conscious freely behaving rats, either before treatment (n = 11) or with partial (aortic) deinnervation of baroreflex afferents (n = 10) in order to enlarge the range of variation of the sympathetic baroreflex sensitivity. The transfer gain at mean arterial pressure was 0.8±0.2 mmHg (95% CI 0.5-1.0) 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 < 0.001) indices of arterial baroreflex sensitivity. In control rats, the transfer gain exhibited large fluctuations (variation coefficient = 34 ± 5%) that were not consistently related to changes in the mean level of arterial pressure, heart rate, renal pressure activity and sympathetic 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.

421 Activation of AMP kinase alpha1 subunit induces aortic vasorelaxation in mice

Introduction: Vasorelaxation is a vital mechanism of systemic blood flow regulation. Arterial baroreflex activity occurs during baroreflex-mediated systemic cardiovascular changes, and is associated with the AMP-activated protein kinase (AMPK) as the major hepatic and adipose tissue serine-threonine kinase involved in energy homeostasis. This study was designed to investigate AMPK activity in aortic rings of normal and obese rats. The effects of AMPK activator AICAR were studied in vitro for their possible effects on aortic rings.

Methods: Three groups of male rats were used: control (C), 5/6 nephrectomy (N), and AMPK activator (AICAR; 100 mM for 30 min). Significant differences were found in aortic rings in response to aortic baroreceptor deinnervation (paired t-test; P < 0.001). AICAR induced aortic relaxation in aortic rings that were completely abolished in AMPK-1–/– but not AMPK-2–/– mice.

Conclusion: Taken together, the results show that activation of AMPK but not AMPK2 is able to induce aortic relaxation in mice, in an endothelium and eNOS-independent manner.

422 Platelet aggregation induced by ADP in rats in vitro

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 were studied in vitro in conscious rats.

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 41.24 and 5.3 mm respectively. For Psammomys obesus, the amounts of adenosine diphosphate for 2.5 μM and 0.6 μM induced an average aggregation intensity of 20.2 and 20.5mm. In the same way and for obese Psammomys obesus, the values of intensity were 40, 43, and 42 mm.

Conclusion: Other research involving made diabetic sand rats will be conducted shortly to assess the effect of various therapeutic agents on platelet function. The sand rat Psammomys obesus appears to us a potentially interesting model for investigation in diabetic syndrome and its effects on platelet function.
of the left middle cerebral artery with a transient occlusion of the common carotid artery, we demonstrated that blood flow velocities only decreased by 15% in the left middle cerebral artery, while blood flow velocities increased in the right carotid artery by 57% and in the basilar trunk by 54%. 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.

425 Determinants of pulmonary artery pressure in valvular aortic stenosis
Introduction: Some patients with valvular aortic stenosis (AS) present with pulmonary hypertension and high transmural/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 heat, chest pain, or palpitations). Left ventricular ejection fraction ranged from 15 to 82%, aortic valve area (AVA) ranged from 0.21 to 1.66 cm² and the TTG ranged from 12 to 65 mmHg. The TTG was higher in symptomatic compared to asymptomatic patients (33 ± 12 vs. 24 ± 8 vs. P = 0.002). TTG was 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 = 0.05), PDE5A (r = -0.27, P = 0.05) and LAA surface (r = 0.21, P = 0.05), the ratio of systolic blood pressure (SBP) over diastolic pressure (DBP) (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 = 0.17 vs. P = 0.17 and r = 0.18, P = 0.11 respectively). By multivariate analysis, independent echocardiographic determinants of TTG were LA surface (P = 0.038), LVEF (P = 0.002) and the ratio of systolic blood pressure over diastolic blood pressure (P < 0.0001).

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 end diastolic pressure and its time-averaged maximum and minimum determinants of TTG in AS. Further studies are needed to confirm these results.

426 Cross-talk between the two thrombin receptors in human endothelial cells
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 stimulates endothelial nitric oxide synthase (eNOS) through a nuclear factor kappa B (NFκB) dependent mechanism. As NFκB is a transcription factor, we postulated that thrombin might also modulate transcriptional activating factors such as AP-1.

Methods: Human umbilical vein endothelial cells are stimulated by thrombin or a thrombin analog to produce the reporter gene luciferase (luc) driven by the AP-1 containing promoter region of the chicken beta-globin gene. As luciferase activity is measured by a simple and rapid method, we decided to use this model to study the cross-talk between thrombin receptors.

Results: Human umbilical vein endothelial cells are stimulated by thrombin or a thrombin analog to produce the reporter gene luciferase (luc) driven by the AP-1 containing promoter region. As luciferase activity is measured by a simple and rapid method, we decided to use this model to study the cross-talk between thrombin receptors.

Conclusion: Cross-talk between thrombin receptors is a complex phenomenon, which can be used to modulate transcriptional activating factors such as AP-1.

427 AMP-activated protein kinase is involved in both NO- and EDHF-mediated relaxations in cerebral arteries
Introduction: Several epidemiological studies have shown that regular consumption of moderate amounts of wine, in particular red wine, is associated with a decreased risk of cardiovascular diseases. The protective effect has been attributed 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 release of nitric oxide (NO). The 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 charybdoxin 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^-nitro-L-arginine in mesenteric artery and coronary artery rings. NO and EDHF do 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 30 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.

428 Marmalade imaging of central artery and vein of the retina as a tool to evaluate perfusion of the retina
Introduction: Spectral analysis of the Doppler signal recorded in the central artery and vein of the retina with colour-coded pulsod Doppler ultrasound imaging enables detection of central retinal artery occlusion (CRAO) and central retinal vein occlusion in patients 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 with central retinal vein occlusion (aged from 40 to 65 years) were studied. Mean flow velocities from CRVO were explored. Times between vein occlusion and the ultrasound study were ranged from 10 days to 720 months. Peak systolic (PSBVCA), end-diastolic (EsBVCA) and time-averaged mean blood flow (tBMF) 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 array transducer (Vivid 7, GE Medical Systems, Horten, Norway). Maximum (MaxBFVcv), minimum (MinBFVcv) and time-average mean (MeanBFVcv) blood flow velocities were measured in the central vein downstream retina. The ultrasound imaging of carotid arteries was performed to rule out patients with carotid stenosis.

Results: In normal subjects, in the central artery, PSBFVcv was 12.9 ± 3.4 cm.s⁻¹, EDRBFVcv 9.1 ± 3.1 cm.s⁻¹, and 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, PSBFVcv was 9.6 ± 3.2 cm.s⁻¹, EDRBFVcv 6.3 ± 2.0 cm.s⁻¹, MaxBFVcv 3.1 ± 0.5 cm.s⁻¹ (P < 0.01 vs. normal subjects), MinBFVcv 1.9 ± 0.3 cm.s⁻¹, and MeanBFVcv 2.7 ± 0.2 cm.s⁻¹ (P < 0.01 vs. normal subjects). There was no difference between values of blood flow velocities in contralateral healthy eye and values in normal subjects.

Conclusion: Measurement 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, upstroke, a recognisable 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.

429 Did ACE inhibition protect skeletal muscle metabolism from acute ischemia-reperfusion effect?
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Vascular "Steinere"

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 (n=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 25 consecutive days by 40 mg/kg/day of captopril. Each experimental animal was dissected at the end of the experimental period and then 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. Mitaximal oxidative capacities of the muscle from the left limb and contralateral right limb, 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 mmol O₂/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 mmol O₂/min/g dry weight respectively P = 0.006). There is a 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 (complexes I -55%, II - P = 0.002; complex I: -50%, P = 0.0015) 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. The administration of ACE inhibitors protected the skeletal muscle from ischemia-reperfusion, but it could be interesting to study the recovery of muscular oxidative function on a midterm and long term basis.

430 Induction of angiogenic factors in skeletal muscle of rat by low-voltage electrical stimulation

Introduction: Promotion of angiogenesis is vitally important after acute arterial occlusion. Vascular endothelial growth factor (VEGF) is the major enhancing factor of vessel growth. According to some recent reports the VEGF production in skeletal muscle could be promoted by electrical stimulation (ES), but the mechanisms involved are not fully elucidated. In hind limb ischemia model of rats, we studied whether LIVES 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 used. According to some recent reports the rats were kept on a specific commercial diet. Before the measurements, mdx, sjl and control tissues were dissected and rapidly conserved in Kreb solution.

Results: On one hand, we observed a large increase of the attenuation and thickness in dystrophic-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 vascular and structure abnormalities in dystrophic mice. In these pathologies, is also distinguishable by such micro-acoustic methods when applied to muscle from older animals.

432 Peripheral venous distension and susceptibility to hyperventilation-induced central apnea in ashenated 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 capillary 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 hypoxia-reduced-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 central mechanisms of CHF) to the propensity for hypoxia-reduced-induced central apnea. We used specific high frequency transcutaneous induritor (20–80 MHz) has been designed. It was tested on various muscles (diaphragm, pectoral, abdominal) to detect any differences.

Methods: Thanks to this specific device, 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.

Conclusion: This work shows the feasibility of this new method in improving membrane evaluation. The aim of this work is to use this method to compare biophysical properties after specific therapeutic treatments. Now, we plan to investigate whether the muscle degeneration process, particularly severe in these pathologies, is also distinguishable by such micro-acoustic methods when applied to muscle from older animals.

433 Reduction in histamine-induced lung ventilation heterogeneity by PEEP: a synchronron radiation computed tomodonography (SRTCT) study in rabbit

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 SRTCT 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 H₂O, at baseline and 16 min following histamine (125 mg/ml) aerosol administration. Ventilation heterogeneity was measured as the difference in mean ventilation between the right and left lungs using SRTCT imaging. 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 after PEEP (5 cm H₂O) and 2 min after mean ventilation.

Conclusion: A PEEP of 5 cm H₂O reduced regional ventilation heterogeneity due to histamine-induced broncho constriction. Such a finding could be useful on SV. Further experiments are underway to confirm this effect and to elucidate its mechanisms.
436 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 (FEV1), peak expiratory flow (PEF), maximal expiratory flow at 50% of FVC (MEF50) and maximum mid expiratory flow between 25% and 75% (MMEF25–75) were measured with a portable spirometer (Minato) in 764 asymptomatic, non-smoking, healthy Tunisian children aged 0.5–18 years.

Results: 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) by 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.

Conclusion: The adjusted risk to have a low pulmonary function was divided by around 2 by additional year: OR = 0.8 (0.8; 0.88) P < 0.000 0001, by additional centimeter. The major and protective effect of the age (or the height) held out, the adjusted expression of aggravating risks showed that predictive factors linked to low pulmonary functions were: female sex for FVC and PEF (OR = 2.0) and 1.67 and P < 0.001 and 0.01 respectively), low birth height for FVC (OR = 1.52, p = 0.01), and for PEF (OR = 1.54, p = 0.008 and 0.001 respectively), and gas heating vs. electricity and outdoor pollution for MMEF 23–75 (OR 1.92 and 2.40, P < 0.0008 and 0.02 respectively). All patients of the study had normal force expiratory volume loops. Curved indices 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 described a different structure.

437 Effect of active sensitisation to Dermatophagoides pteronyssinus allergen on respiratory function in brown Norway rats

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Introduction: The aim of this study was to develop and characterize a model of allergic 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 (100 µg). At days 1 and 3, (D1 and day 3) (D0), followed at D17 by intratracheal instillation of D pter. Control (C) rats (n = 5) underwent the same protocol but with saline solution (0.9% NaCl) during 24 h. Histological and morphometric observations were made at the end of the experiment. At days 3 (D3), enhanced expiratory pause (Penh), used as an index of airway resistance, was measured using a barametric plethysmograph for conscious animals (Buxco, Troy, NY, USA). At the bronchial segments, the mechanical contractile was measured on rings isolated from trachea (T), extrapulmonary (EPB) and intrapulmonary bronchi (IPB) using an organ bath system (EMKA, France). Maximal contraction (Cmax) and IC50 were derived from cumulative 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 transcverse study concerning 42 OSAS diagnosed by PSG of the same snorers and 15 non snorers. OSAS was measured by using a plethysmograph and a NEP technique performed in the seated and supine positions in a random order. The depression was limited to 5 cm3 O2.

Results: All patients of the study had normal force expiratory volume loops. Apnoeic patients had lower Dflow in both positions with a number of those oscillations on the expiratory curve obtained with NEP and an EFL in supine position was raised in all subjects (p < 0.05). Passage from the sitting to the supine position raised Dflow 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 strong correlation was found between apnoea-hypopnoea index and Dflow and between Dflow and IAHI especially in supine position (p < 0.05). The analysis of variance showed that the difference was significant between the 3 groups of the study concerning DNE and number of oscillations with respective sensitivity of 83% and 90%. Post - hoc comparison showed that only the number of oscillations was increased significantly differently between the three positions.

Conclusion: NEP constitutes a simple and useful field for the screening of 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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439 Simplified assessment of insulin sensitivity (SI) over 2 h after a standardized hyperglycemic breakfast (SHB)
JF Brunot1, E Raynaud2, J Mercier1* Montpellier - France
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 insulin sensitivity (IVGTT)] recent studies have emphasized the accuracy of a simpler approach: the minimal model analysis of a standardized hyperglycemic breakfast. In this "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 glucose tolerance test (IVGTT). In Shaour’s studies we noted that the diameter colloid batch insulin is smaller than the other three batches. The treated batches of the first and of the second group were sacrificed thirty five days later and the other forty five days. The third group is treated by dextrose 6 mg/kg BW of streptozotocin during thirty days and the last group is daily treated by the insulin 200 i.u/kg BW.

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.

Conclusion: Our data consolidate the importance 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, and the immunizing response to the infections. It was reported that a deficit in energy, protein, iron, copper, vitamin A, C and B6. The available data in the literature, concerning notably the effect of the hypothyroidism on the profile lipidique and lipoprotéique, and its relation with the atherosclerose seems to be undeniable. In Tunisia, the struggle against the improvement of the micronutrients deficiencies has always constituted a priority of the Ministry of Health.

441 Biologic profile in tunisian infants hospitalized for malnutrition
M Kamil1, R Aouad2, R Srairi2, A Aoudiet* Sfax - Tunisie Manouba - Tunisie Tunis - Tunisie
Conclusion: 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 always a problem preoccupying in public health and notably when it reaches young children less than five years. Malnutrition affects various significant functions such the physical growth, the mental development, the capacity of training and the immunizing response to the infections. It was reported that a deficit even a mild in energy, protein, iron, copper, vitamin A, C and B6. The available data in the literature, concerning notably the effect of the hypothyroidism on the profile lipidique and lipoprotéique, and its relation with the atherosclerose seems to be undeniable. In Tunisia, the struggle against the improvement of the micronutrients deficiencies has always constituted a priority of the Ministry of Health.

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spingolipid 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 cardiacomyocytes were enzymatically isolated, and electrophysiological studies were done with patch clamp technique, in whole cell configuration. Neonatal cardiacomyocytes maintained in culture were transfected with 0.5 μg of GFP-tagged Kv1.5 expression vector using FuGENE® 6 Transfection Reagent. These results suggest that the protein subunit distribution determine 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_{Ks}, which reached steady-state in ~7 minutes. This effect was prevented by predosing the MCD with cholesterol. MCD-increased current was abolished by 500 μM 4-AP. Neonatal rat cardiacomyocytes transfected with GFP-tagged Kv1.5 channels showed a large ultrarapid delayed-rectifier current (I_{D}), which was also stimulated by MCD. In atrial cryosections, Kv1.5 channels were located in the intercalated disc at the surface membrane. MCD caused reorganization of Kv1.5-subunits to clusters in the low-density fractions of step sucrose-gradient preparations. In live neonatal cryosections, Kv1.5 channels were found at the intercalated disc, whereas clusters were predominantly organized in the cytoplasmic 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 in the sarcoplasmic reticulum 

**Introduction:** Expression of TRPC3 / 6 in human jurkat T cells

**A Ple´**

**Expression of TRPC3 / 6 in human jurkat T cells**

**A Ple´,** A Hichami*, NA Khan* "Dijon - France"

**Introduction:** In this study, we investigated the presence of mRNA, encoding for two members of transient receptor potential (TRP) calcium channels, TRPC3 and TRPC6, in human jurkat T cells. These channels are activated by fatty acids and, particularly, by diacylglycerols (DAG).

**Methods:** We employed RT-PCR technology in order to identify TRPC3 / 6 in human jurkat T cells before and after stimulation with different mitogens like phorbol 12-myristate 13-acetate (PMA) plus ionomycin and anti-CD3 antibodies which exert different mechanisms of action in the induction of T cell proliferation. The T cells are rendered quiescent by overnight incubation in the absence of serum and then stimulated by these mitogens.

**Results:** Correlation between transcription of mRNA and protein expression in these cells. We observed that there existed a corelation between the expression of TRPC3 / 6 mRNA and II-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 expressed at 2 hours of stimulation by alone 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 aforementioned observations.

**Expression of mRNA for RhoA-guanine exchange factors is modulated by angiotensin type 1 receptor in rat aorta smooth muscle cells**

D Ferland-Mccollough*, C Cario-Toumaniata*, G Toumaniata*, G Lorient*, P Paucourt*, J Rivet* "Dijon - France"

**Introduction:** Angiotensin II (ANGII) has been implicated in various cardiovascular diseases. Therefore, characterization of the ANGII type 1 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 signaling 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 expression 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) isolated from Wistar rats were cultured in 10% FCS-containing RPMI with 100 μM 4-AP. ASMCs deprived in serum 24 h. GEFs mRNA expression levels were analysed in cultured ASMCs incubated with 1 μM of AT2R antagonist PD123,199, with or without 100 μM losartan for 1, 4, 12 or 48 h in the presence of absence of losartan (1 μM), an AT1R antagonist, and fasakiil (1 μM), an inhibitor of Rho-kinase.

**Results:** RhoA-GEF expression profile analysis revealed three groups of distinctively regulated GEFs. The first group is composed of 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 composed of four GEFs which showed a low expression level. Obscursin was not detected in cultured ASMCs. Transcriptional modulation of RhoA-GEFs by ANGII-stimulated AT1R was observed at 1, 6, 12 and 48 hours of stimulation. ANGII-induced expression changes occurred transient variations of few RhoA-GEFs (scambio, p190, CDEP, n = 5), an early (1 h) and maintained down-regulation of the RhoGef 6 (n = 5), and a late (48 h) down-regulation of a group of 9 RhoA-GEFs mRNA including adiletteur, 1, 2, 11, 12, 18, BCR, BCT2, p61RhoGEF and vase2. 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, fasakiil was tested and we observed a loss of GEF-mRNA down-regulation except for p61RhoGEF and Arti1 (n = 5).

**Conclusion:** These results confirm that the Rho-kinase-modulated ANGII expression profile in ASMC and show that RhoA-GEFs are transcriptionally modulated by AT1R-AngII stimulation through RhoA/Rho-kinase dependent and independent pathways. These observations shed light on the protein complex feedback mechanisms between ANGII-induced RhoA activation and the transcriptional regulation of its activators.

**Effect of specific respiratory muscle training in mdx mouse on cardiac mitochondria and microdomains**


**Introduction:** The cellular mechanisms resulting from the low exercise 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 (% CO2), 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 (14.1 ± 0.4 vs. 9.5 ± 0.3 μmol/min/mg) with no difference between the groups in diaphragm muscle fiber type and in citrate synthase activity. In addition, ryanodine receptor (RyR) activity was the extraplated on individual diaphragm fibers using laser scanning confocal microscopy. It showed decrease of Ca+2 spike-rise-time in training group (4.66 ± 0.11 vs. 5.39 ± 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 may be partially explained by an improvement of mitochondrial function and RyR gating properties which could attenuate the basal rise of cytosolic Ca2+.

**Regulation of TRPC-dependent calcium influx by dystrophin/a-syntrophin complex: implication in Duchenne muscular dystrophy**


**Introduction:** Duchenne muscular dystrophy is a neuromuscular disease which leads to the progressive degeneration of skeletal muscle fibers. Major causes of muscle dysfunction are the lack of dystrophin, a protein located at the cytoplasmic face of the sarcolema. In normal skeletal muscle cells, dystrophin is associated with actin and calpain complex (DAP) and with caveolin (caveolin is the specific coregulator of caveolin-3). 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 SOCE (Store Operated Channel) carried by TRPC1/TRPC4.

**Methods:** The aim of our study is to identify the proteic environment of SOCE by specific precipitation technique and to investigate the 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 a-syntrophin through its PDZ domain. This last protein, which is a scaffolding protein, could mediate a molecular link between the dystrophin based cytoskeletal network and TRPC1/TRPC4. This complex is associated with a-syntrophin from mdx muscle than from normal muscle. The decrease of this complex is likely due to the reduction of a-syntrophin at the sarcolema in dystrophic muscle cells. This interaction could be the main cause of SOCEs dysfunction (Store Operated Calcium Entries) by overactivation of SOC. To explore the functional regulation of SOCEs by a-syntrophin, experiments were designed to overexpress a-syntrophin in cultured myotubes. The measurement of calcium influx by quenching of fura-2/AM by manganese showed that forced expression of a-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 a-syntrophin could be essential for maintaining a normal regulation of SOC activity. The expression of a-syntrophin could be a therapeutic approach that needs to be considered. Pharmacologic step in order to specifically inhibit TRPC-dependent SOCE could show a direct correlation between this inhibition and regression of cellular death observed in dystrophic mouse.

**Aggregation of vascular myocytes to microgravity by the decrease of ryanodine receptor subtype 1 expression**

449 Evidence for functional coupling between mouse cardiac fibroblasts in primary culture
C Louault*, J Bescond*, D Piret* *Périers - France

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 interaction was determined by measuring the dye fluorescence ratio (fluorescein vs. DAPI) by Photobleaching (gap-FRAP) method with carboxy-fluorescein as dye. To be able to observe fibroblast differentiation into myofibroblast, αSMA-RFP mice which express red fluorescent protein under the control of the promoter alpha smooth muscle actin, a protein specifically expressed in differentiated myofibroblasts, 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 in vitro culture. The rate of communication is decreased in the presence of Ca²⁺ with a threshold of permeability k = 6.0 ± 0.5 10⁻²⁵ min⁻¹ in fibroblast culture [n = 65] increased to k = 14.6 ± 2.8 10⁻²⁵ min⁻¹ in myofibroblast cultures [n = 109]. We also observed 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-RFP mice that show fibroblasts deeply differentiate into myofibroblasts during cell 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 express connexins 40 and 43 which are able to establish functional communications between cells and to form a coupled network. The amplitude and efficiency of this coupling could be functionally related to the state of fibroblast activation, which will be of particular interest in the perspective of cardiac cellular therapy.

450 Myopathies with dystrophin deficiency: evidence for the involvement of IP3 calcium channels in the calcium deregulation and effect of IP3 blockers
L de Monchy*, I Balghith, B Constant*, C Cognard*, S Sebbale* *Périers - France

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 of such calcium release could restore a non-pathologic context, cell survival assays (MTT test) were also performed in IP3 pathway attenuated conditions.

Results: Comparison to controls rats receive injections of NaCl with 0.9% (n = 10). The incubator has two compartments, one exposed to microgravity and the other not, for a mean of 6 days (± 2). The influence of hyperhomocysteinemia on blood lipids, lipoproteins and vascular disease risk. It has been reported that hyperhomocysteinemia is associated to cardiovascular diseases and is responsible for the myocardial infarction is a completely occlusive thrombosis of a coronary artery. Here we analyze the mechanisms of RhoA regulation by serotonin (5-HT) in arterial smooth muscle.

Conclusion: Transamination of αSMA leads to RhoA activation and enhanced proteasomal degradation, which in turn is responsible for Akt activation and contraction inhibition. The observation of transamination of 5-HT to RhoA in culture of twitch myocytes from mice suggest that this process could participate in pulmonary artery remodeling and hypertension.

451 Transglutaminase-dependent rha activation and depletion by serotonin effects on vascular smooth muscle
P Zerrer*, K Othmani-Mecif, L Raul*, Y Benazzoug*, N Rekkallah*, S Aouchit-Bougouer*, M Cherif* *Alger - Algerie

Introduction: Following an acute coronar occlusion, the first cellular lesions characterized by ischemic 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 ischemic myocardic mass. In 90% of the cases, occlusion perpetuated, the myocardic 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 (% of egg yolk cit) and a pilot batch (n = 9) subjected to the halophylous plants (50 g/l) 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 cholestrolémia, triglyceridémia and glycemie after only 1 month of hyperlipidic diet (1824 ± 661 mg/dl, vs. 48 ± 2 mg/dl), (649 ± 423 mg/dl vs. 26 ± 2 mg/dl), (117 ± 82 mg/dl vs. 43 ± 10 mg/dl). A significant increase in the rate of the troponine I (560 ± 229 U/l vs. 273 ± 101 U/l), (0,09 ± 0,02 µg/l vs. 0,12 ± 0,09 mg/l) respectively. The morphological examinations of the heart revealed beaches of significant ishaemica necroses and some infarctes arrests.

The mycardic cells in culture (early passage), resulting from animals subjected to the hyperlipidic diet show after 72 H of incubation (10⁶ cells/ml), a reduction in both the rate of their proliferation which reaches 28% vs. 113% their corresponding witnesses.

Conclusion: In conclusion, experimental hyperlipidemy in Psammomys obesus armature of the metabolic deteriorations, marked by an increase in the cholestrolémia, triglyceridémia and glycemie, 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 ishaemica necrose followed by an infarction localised.

453 The influence of hyperhomocysteinemia on blood lipids, lipoproteins and vascular disease risk
P Zerrer*, K Othmani-Mecif, L Raul*, Y Benazzoug*, N Rekkallah*, S Aouchit-Bougouer*, M Cherif* *Alger - Algerie

Introduction: Here we analyze the mechanisms of RhoA regulation by serotonin (5-HT) in arterial smooth muscle.

Methods: Here we analyze the mechanisms of RhoA regulation by serotonin (5-HT) in arterial smooth muscle.

Results: 5-HT (0.1—10 µM) induced maximal activation of RhoA followed by RhoA depletion at 24—72 h. Inhibition of 5-HTI receptors reduced the early phase of RhoA activation but had no effects on 5-HTII receptor-induced RhoA activation and depletion, which were suppressed by the 5-HT transporter inhibitor fluoxetine, the transglutaminase inhibitor monodansylcadaverin and in type 2 transglutaminase-deficient smooth muscle cells. Immunoprecipitations demonstrated that 5-HT associated with RhoA both in vitro and in vivo. This association was calcium-dependent and inhibited by fluoxetine and monodansylcadaverin. 5-HT promoted the association of RhoA with the E3 ubiquitin ligase Smurfl, and 5-HT-induced RhoA depletion was inhibited by the proteasome inhibitor MG132, and the RhoA inhibitor Tatt-C1. 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-HTII-mediated RhoA degradation by MG132 prevented 5-HT-induced Akt activation. Long-term 5-HT II 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 transaminated to RhoA by transglutaminase.

Conclusion: Transamination of αSMA leads to RhoA activation and enhanced proteasomal degradation, which in turn is responsible for Akt activation and contraction inhibition. The observation of transamination of 5-HT to RhoA in culture of twitch myocytes from mice suggest that this process could participate in pulmonary artery remodeling and hypertension.

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Results: A moderate hyperhomocysteinemia (20.09 ± 7.77 µmol/l) is observed in the sixth month of treatment against 2.63 ± 2.01 µmol/l at the beginning of the experimentation. Both cholesterol and triglyceridemia undergo variations during our experimentation. The analysis of the lipodrograms highlights a significant decrease in the IH group, contrary to the LH group, and markedly less from the B300 group. The Psammomys obesus hyperhomocysteinemic is the seat the many focused deteriorations represented by blood aggregations luminales, a hypertherpy of the endothelium and subendothelium, ruptures and unfolding of the internal elastic lamina, plates of fibrinoid necrosis and interstitial inflammatory cell infiltrate. Light 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 can confirm that hyperhomocysteinaemia 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 elucidated.

**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 Biotec, Germany). The isolated cells were cultured for 24 h in 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 increased in free intracellular calcium concentrations, [Ca++]i, via the activation of FAT/CD36. SulfO-N-Sucinimidyloleate, an inhibitor of FAT/CD36, abolished LA-induced increases in [Ca++]i. We have also observed that LA increased the expression of tyrosine residues of Fyn and Yes which belong to the family of Src kinases. Furthermore, the inhibitors of tyrosine kinases (PI2, Genistein and SU 6656) and an inhibitor of SOC (Soc2 operated calcium channel) increased the elevation of [Ca++]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++]i in murine CD36-positive cells from circumvallate papillae.

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**Role of RYR1 splice variants in calcium signalling in mouse myometrium during pregnancy**

F Dabretrand1, N Frites2, N Macrez2, JL Morel3 * 4Bordeaux - France 5Stockholm - Sweden

**Introduction:** Alternative splicing of the RYR1 tyrosine receptor subtype generates a RYR1S short isoform without channel function and a functional RYR1L full length isoform. It has been previously shown that the RYR1S isoform could negatively regulate the native RYR2 subtype in smooth muscle cells as well as the RYR3 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-ribose 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 and localization of RYR1 splice variants were specifically inhibited by electroporation of antisense oligonucleotides. The inhibition of RYR3 isoforms was evaluated by RT-PCR, RYR3 immunostaining and western blot in non-pregnant and pregnant mouse myometrial cells. Calcium transients induced by cyclic ADP-ribose was measured by fura-4 and laser scanning confocal microscopy.

**Results:** Here, we show that both isoforms of RYR3 are expressed in non-pregnant and pregnant mouse myometrial cells. The use of LA-induced increased cyclic ADP-ribose against each isoform indicated that only RYR3L was activated by caffeine and by cyclic ADP-ribose and that RYR1L-mediated Ca2+ release was negatively regulated by RYR1S expression in non-pregnant myometrium. At the end of pregnancy, both expression and activity of RYR1L 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-phenoxycetic acid (2,4-D) on parameters of reproductive function in male rats**

D Cherif1, M Lakhdari1, A Ben Romdhane - Tunisia

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 drainages 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 2,4-D on the reproduction. One the 2,4-D on 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 relative weight of the LH and FLH and FSH (luteinizing hormone) (testosterone) was also determined. 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. 202.6 ± 4.4 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 7.0% (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.

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**How to determine glomerular filtration rate (GFR) before liver transplantation?**

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**Introduction:** Drug-induced nephrotoxicity during 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 methods to estimate glomerular filtration rate is of great concern since there is no gold standard test responsible for a decrease in muscle mass and often associated with jaundice that interferes with the classical Jaffe's method for creatinine dosage. Our aim was to determine (i) if creatinine-based methods are 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 technique after a post-prandial meal. 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 Cockcroft-Gault Formula (CC), 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 NFLs (K/DHQ) 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 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 NFL misclassification, included the lowest lowest GFR value.

**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 African plants termed A and B respectively were prepared either by organic or aqueous solubilization. Extract A was obtained after 24 h solubilization in a mixture CH3Cl/MeOH/ H2O 33% DMSO and is administered at the dose of 75 (A75) and 100 mg/kg/day (A300). Extract B is an aequous solution and is administered at the dose of 150 (B150) and 300 mg/kg/day (B300). Both extracts were given by gavage to Wistar male rats made diabetic by a single administration of streptozocin for 14 days. Non diabetic C57 BLks db/m were treated both by gavage and by adding them to the diet. The body weight and of both extracts to STZ-diabetic rats resulted in complete correction of hyperglycemia. The values of glycemia (mg/dl) were A75 = 108 ± 12; A300 = 155 ± 12; B150 = 1.3; B300 = 1.2; at the beginning of the experiment and B75 = 75 ± 12 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 effectiveness of these treatments in type 2 diabetes using RIKS db/db mice.

459 Renal localization and expression of tight junction proteins, Claudin-2, Claudin-3, Claudin-5 and Occludin in the rat kidney.

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Introduction: In the recent years, there have been important advances in the field of molecular biology that have allowed a better understanding of the expression, function and regulation of tight junction protein complexes in the kidney. Claudin family proteins have been reported to be expressed in the rat kidney. However, the localization and expression of Claudin-2, Claudin-3, Claudin-5 and Occludin in the rat kidney have not been reported before. The present study examined the renal distribution of these Claudin family members in the rat kidney.

Methods: Kidneys from 2, 7, 14, 21 and 60 day old Wistar rats were perfused with the fixative and samples were fixed in Bouin's solution or in 4% paraformaldehyde. After dehydration and embedding in paraffin, tissue sections were stained with hematoxylin and eosin. Immunohistochemistry was performed with monoclonal antibodies against Claudin-2, Claudin-3, Claudin-5 and Occludin. Sections were incubated with primary antibodies overnight and then with secondary antibodies conjugated to biotin and horseradish peroxidase. The tissue was counterstained with hematoxylin.

Results: Claudin-2, Claudin-3, Claudin-5 and Occludin were localized in the tubular epithelium, renal blood vessels and glomerular capillaries. Claudin-2 was expressed in distal tubules, renal blood vessels and in the renal interstitium. Claudin-3 was expressed in proximal tubules, renal blood vessels and in the renal interstitium. Claudin-5 was expressed in distal tubules and renal blood vessels. Occludin was expressed in proximal tubules, renal blood vessels and in the renal interstitium.

Conclusion: This study provides new information about the renal localization and expression of Claudin-2, Claudin-3, Claudin-5 and Occludin in the rat kidney. These data will allow a better understanding of the role of these tight junction proteins in the renal physiology and pathophysiology.

460 Barin growth in autism: first study of evidence of prenatal overgrowth

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Introduction: Autism is a complex neurodevelopmental disorder that affects approximately 1% of the population. The etiology of autism is not fully understood, but genetics and environmental factors play a role. Several studies have suggested that the timing of fetal growth is important in the development of autism. The aim of this study was to investigate the prenatal growth of children with autism.

Methods: The study included 48 children with autism spectrum disorder (ASD) and 48 typically developing children (controls). The children were divided into two groups based on their age. Prenatal ultrasound measurements of biparietal diameter, head circumference, abdominal circumference, and femur length were obtained at 20, 24, 28, 32, and 36 weeks of gestation. The data were analyzed using regression analysis to determine if there were significant differences in fetal growth between the two groups.

Results: The biparietal diameter and head circumference were significantly larger in the autism group compared to the control group at all gestational ages. The abdominal circumference and femur length were also larger in the autism group, but the differences were not statistically significant.

Conclusion: This study provides evidence of prenatal overgrowth in children with autism. Further studies are needed to confirm these findings and to investigate the underlying mechanisms.

461 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 spinal equina. This study evaluates the changes in M wave and H reflex. 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 nerves than on motor nerves. This effect of lidocaine on the reflex pathway has not been expressed in the literature. To confirm it, we propose an animal study of the time course of H reflex and M wave after lidocaine injection.

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. The animals were then stimulated in the hind limb (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 the 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 injection of lidocaine, the stimulation was applied every minute and until 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 (+/-0.5 ms) for H reflex. The mean maximal peak to peak amplitudes were 36.4 mV (+/-1.1 mV) for M response, and 13.7 mV (+/-0.7 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 5% (+/-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 H reflex was confirmed that lidocaine seems to be more efficient on the sensory spinal afferents than on the motoneurons. The lidocaine blockage therefore appears very interesting in spasticity assessment. Concerning these experiences are in progress to better understand the mechanisms of lidocaine action.
Conclusion: When scaling with the RS: X board, physiological demand seems to be higher in the presence of the official Observer windrow board [mimical one design® (MOD)]. This difference could be mainly attributed to the specific biomechanical constraints induced by each board characteristics.

4.6 Physical training and evoked potentials: neurophysiological particularities in sports involving vision

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Introduction: The purpose of this study was to investigate the tracking neurophysiological particularities in sportmen using evoked potentials, and more specifically at assessing how sports involving vision influence evoked potential patterns.

Methods: Twenty sportmen and ten sedentary subjects participated to this study. Sportmen 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, sportmen 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 sportmen for earlier components: N1, P2 and N2. Tenennismen demonstrated shorter visual P300 latencies than shooters, without latency differences in the P101 or P104 components and in the P300 latency. No significant differences were found in latencies between sportmen and sedentary subjects for visual evoked potentials nor for the visual P300 component. In the auditory component, only tennennismen differ from sedentary subjects with shorter latencies in brainstem auditory evoked potentials (for the I-V interwave interval, and more specifically the IBV interwave interval). No other significant differences were showed between sportmen and sedentary subjects in amplitudes neither for brainstem auditory evoked potentials nor for the auditory P300 component.

Conclusion: The present study suggests that specific visual and dynamic vision might influence electrophysiological data in the visual modality in different ways at cognitive level, without differences at sensory and perceptual level. Moreover, the sensitivity is also associated to electrophysiological particularities in the sensory test. The visual modality is shown to depend at the P300 level from the auditory modality since the latter is critical in this sport. Present findings argue for neurophysiological particularities in sportmen related to sensorial skills and cognitive abilities needed by their physical training.

4.6.1 Skeletal muscle recovery after notoxin injury: positive effects of running exercise

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Introduction: Skeletal muscle is able to regenerate after extensive injury (notoxin injection) probably through fast activation and proliferation of satellite cells. Then new myocytes differentiate into myoblasts and proliferate to form foci of immature myotubes and immature MHC isoforms. During maturation, myotubes fuse to form mature myocytes with peripheral nucleus and expression of mature MHC isoforms. The recovery of muscle mass takes more than 40 days in sedentary animals. When physical activity is increased (28 days suspension during recovery), the regenerated muscle mass is also increased. It is hypothesized 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 rats were injected with Notoxin and right ones were kept as control (D0). Three days after injury, half of the animals began running exercise: calibrated exercise on treadmill associated with voluntary (exploratory activities) and sedentary (E). The other half (D1) was kept in the 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 non-intact). 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 values of the two sub-groups were similar (respectively 33.3 ± 3.7 years; 24.5 ± 2.1; 31.7 ± 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 on 30, 60 and 90 days after.

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 relation between the VO2max values and bubble formation.

Conclusion: As age simultaneously influences the VO2max 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 VO2max value. Nevertheless, it is still true that VO2max reflects a subject’s level of physical activity, which is known to influence bubble formation.

4.6.2 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 not in cardiologic patients. In cardiac diseases, which have been associated to a cognitive impairment, research about cognition improvement assumes a clinical interest. We examined the effect of acute exercise and exercise 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 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 to a moderate level during exercise as compared to normal conditions, which enhanced the positive effect of acute exercise on cognitive performances.

Conclusion: In conclusion, exercise training improved both performances among patients with CAD and HF and enhanced the positive effect of acute exercise on cognitive performances.

4.6.3 Biological 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 (1.9 ± 0.8) at the rate of 4 times a week. The control group was formed by 41

4.6.4 Impact of acute exercise on QT dispersion

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Introduction: Exercise tests are usually used for diagnosis of cardiac abnormalities. QT 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-minutes rest with 12-Leads ECG recording each minute. Three groups of patients were defined according to STd criteria: Acute (STd ≤ 4 mm.), Positive (STd ≤ 3 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 STd (P < 0.001) without significant differences between groups. Moreover, all ventricular repolarization parameters decreased deeply during recovery (P < 0.001). Finally, QTd in the control group were different to QTd in the delayed repolarization were shorter than during the resting period (P < 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.
470 Exercise calorimetry with 6 min steps closely predicts the lipid oxidation flow rate in a 45 min steady state body load 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 targeting of training on metabolic bases. We aimed at investigating whether the maximal flow rate of lipid oxidation (LIPOXmax) could be predicted with this step test procedure. We performed a LIPOXmax test of 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 maximal lipid oxidation (LIPOXmax) duringLIPOXmax test. The power corresponding to this LIPOXmax, 11 sedentary subjects (age 49 ± 4.9 years; BMI: 29.4 ± 1.74 kg/m²) 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 calorimeter with an average deviation of 4.51 ± 8.7 mg/min on the Bland-Altman plot and a satisfactory correlation (r = 0.885 ± P < 0.001). Furthermore, the graded calorimetry test predicts the heart rate over the 45 minutes with a 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 appropriate procedure for targeting training on a metabolic basis.

471 VO2, kinetics and bronchial hyper-responsiveness in professional cyclists

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Introduction: The relation between oxygen consumption (VO2) and work rate (W) is classically considered to be linear (Astrand). Recently, the comparison of the VO2 measured during an incremental test with the extrapolated values from the VO2/W relation below the lactate threshold can show an excessive VO2 in healthy subjects (Zoladz) or a reduced VO2 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 pharmacological tests are not always discriminatory enough for a diagnosis. Could the analysis of VO2 kinetics below and above VT provide additional information about Client: the VO2 measured during an incremental test with the extrapolated values from the VO2/W relation below the lactate threshold can show an excessive VO2 in healthy subjects (Zoladz) or a reduced VO2 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 pharmacological tests are not always discriminatory enough for a diagnosis. Could the analysis of VO2 kinetics below and above VT provide additional information about

Methods: A total of 37 professional cyclists (27 ± 4.1 years, 179.7 ± 6.8 cm, 71.2 ± 5.5 ± 9.9 kg, 41 ± 7.4 kg) were separated in two groups characterized by positive (BHR+, n = 18) or negative (BHR-, n = 19) bronchial hyper-responsiveness according to the following parameters (Emmel and colleagues). Lung function test at rest and after exercise and methacholine challenge. Subjects performed an incremental test (50 W/min) until exhaustion on a bike ergometer (Lode Excalibur). Physiological parameters were measured continuously (Airtrac, Jaeger Oxycon) during the test and after exercise and methacholine challenge. 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 ± 1.9 for BHR+ and BHR-; P < 0.05), there was no significant difference between groups regarding anthropometrics, physiological parameters or VO2 kinetics below the lactate threshold. On the other hand, the VO2 relation of the BHR+ group exhibited a breakpoint with a steeper VO2/W slope above compared to below VT (0.497 ± 0.031 vs. 0.561 ± 0.051 respectively, P < 0.05) which was not observed in the BHR- group. The VO2/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 BHR+ and BHR- according to their BHR status. However, it appears that the VO2 kinetics during an incremental exercise test can be a discriminatory parameter contributing to the diagnosis of BHR.
67.125 kg ± 6.65 on average and their size of 177 cm ± 5.90. The age of subjects with HBASA was aged from 25 to ± 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 75% of their theoretical maximum (P < 0.005) on 110 subjects HBAS. The urinary ions had not presented any significant difference in the Na+/K+ subgroups. The subjects 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 thermoregulatory comparable to normal subjects, at rest and after three and five minutes of recovery.

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**475 Determination of caloric contribution and study of deviation of the food behavior in 100 teenagers high school students**

R Aouadi†, Y FekiR Tunis - Tunisia

**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 obesity. The benefit 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 20 to 26 years and weighed 67.21 ± 9.60 kg. This process varied from 3 days per week and for 1 h per day during 8 weeks (1 day per week and 1 h per day) in three different deprived sport halls, with a physical aerobic training program. At the beginning (t0) and to the end (t8) 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 Crompton).

**Results:** The results showed that aerobic physical activity for 8 weeks reduces the body weight only in group G2 without significant modification in their VO_{2} max. However, our findings demonstrated that the subjects of group G1 performed significantly their VO_{2} max but without significant modification in their body weight (the decrease of body weight was with low levels). 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.

**Conclusions:** The relevance of the classification of groups of teenage sprinters according with the federal classification (minim, cadet) and to take in consideration the evolution of this process varies from a person to another. Biologic maturation through the pubertal stages on the exploring strength and the maximal oxygen uptake (VO_{2} max) takes as a basis on the chronological age.

**Methods:** The total sample of our study is composed of 43 runners of speed (aged from 14 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 speed performed on a distance of 300 m.

**Results:** Our data showed that the 22 minimal athletes, aged from 14 to 15 years, were distributed according to the stages to Tanner as five athletes in stade1, 7 athletes in stade1 and nine athletes in stade2. With regard to, the 21 athletes cadets, aged from 16 to 17 years, they were distributed as three athletes in stade1 and 18 athletes in stade2. The evolution, according to the biologic age, of the capacities, the exploding strength and the speed of displacement showed that there was no significant difference between two successive pubertal stages. Indeed, we haven’t noted a significant difference between the stades2 and 1. 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 biologic age rather than the chronological age. This result justifies the 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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**477 Physical activity effects on body weight, VO_{2} Max and heart rate**

R Aouadi†, A Aouidet†, CH Jlid* Tunis - Tunisia

**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:** The purpose of this study was to investigate the influence of physical activity on body weight, VO_{2} max and arterial pressure. The results showed that aerobic physical activity for 8 weeks reduces the body weight only in group G2 without significant modification in their VO_{2} max. However, our findings demonstrated that the subjects of group G1 performed significantly their VO_{2} max but without significant modification in their body weight (the decrease of body weight was with low levels). The influence of physical activity on body weight, VO_{2} max and arterial pressure.

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**Conclusion:** The relevance of the classification of groups of teenage sprinters according with the federal classification (minim, cadet) and to take in consideration the evolution of this process varies from a person to another. Biologic maturation through the pubertal stages on the exploring strength and the maximal oxygen uptake (VO_{2} max) takes as a basis on the chronological age.

**Methods:** The total sample of our study is composed of 43 runners of speed (aged from 14 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 speed performed on a distance of 300 m.

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**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 biologic age rather than the chronological age. This result justifies the 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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**478 Lipid mobilization: a limiting factor of lipid oxidation in trained men**

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**Introduction:** The rate of oxidation of fatty acids (FOx) during exercise 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 surrace ingestion might affect these parameters. Methods: Post-endurance, the rate of FOx during exercise was measured by indirect calorimetry.

**Results:** At rest, lipid mobilization and FOx were significantly higher in Ex compared to Nex. During CON exercise, lipid mobilization 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. During CH0 exercise, lipid mobilization did not increase but remained higher in Ex compared to Nex. During CON exercise, lipid mobilization and plasma fatty acid availability were strong determinants of FOx in endurance trained men.
Methods: In 10 healthy endurance trained runners (mean age ± SD: 19 ± 3 yr), induced sleepiness (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 cells count change significantly. At rest, IS showed prevalence of macrophages (40%) over neutrophils (25.4%); after exercise, total cells increased significantly during the competitive period. Increased neutrophil counts were found in the two last periods of the competition period (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 cells types, our data do not provide a clear-cut role for histamine in the pathogenesis 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.

480 Effect of high-fat diet and physical training on carbohydrate metabolism in rats. N EL El-F, Z Tabka, M Zaouali, A Kamoun, N Gharbi, S El Fazaa. Tunis - Tunisia

Introduction: Physical exercise affects many of both homeostatic mechanisms in which different airways and systemic functions are involved. There are potential benefits 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 (FE; n = 12) and high-fat diet with exercise (FE, n = 12). Animals of 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 on a diet rich in a diet rich in fat. Food intake, weight gain, and body composition 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), glycemia, FFA, and muscle glycogen (P < 0.05). Conclusion: 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.

481 Effect of wingate test on concentration of superoxide dismutase in judo athletes plasma. Z Tabka, K Elbahi, A Lahlhni, I Latiri. Tunis - Tunisia

Introduction: Superoxide dismutase catalyses the dismutation of superoxide anion to molecular oxygen and hydrogen peroxide, and it is likely that they protect cells against lipid peroxidation. The possibility that superoxide dismutase could cause damage including skeletal muscles is upheld by much experimental work. However, if the effect of an acute bout of exercise 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, volunteered 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. Heparinized 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 aspirated 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 antioxidant status was also determined.

Results: Results are shown in table 1. There were significant increase in plasma superoxide 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.

Conclusion: Brief supramaximal exercise induce transient increase in superoxide dismutase in judoka athletes that could protect skeletal muscle from oxidative stress.

482 Swallowing sounds after partial or total laryngectomy. M Boirin, S Morinier, P Reuter. Tours - France

Introduction: We demonstrated that three main sound components (SC) are included in the swallowed sound. The aim of this study was to evaluate the changes in SCs after total (TL) or partial supracricoid (SC) laryngectomy. Patients with neck cancer were examined using our digital acoustic recording technique for evaluating before and after surgery.

Methods: Twenty patients were assigned to the SC1 group (11) or the LT group (9). A microphone was put in place below the cricoid cartilage and connected to the computer. Six recordings were realised before surgery and 6 months after. The COOLEST PRO software was used to sound analysis. The mean number of SCs (N), the mean duration of total sound (SD), the mean duration of all SCs components (SCD) and percentage of percentage for the SC1, SC2 and SC3 for all the recordings were calculated.

Results: SC group. 53 recordings were realised 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 < 0.05). 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. 39 recordings were realised before surgery, and 49 after (2 early dead patients). N before and N after were also not different (2.1 vs. 1.85). SC1 and SC3 were lower after surgery. (SC1 54% vs. 74%; SC3 27% vs. 44%). SD was also 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 msc, 196 vs. 153 msc, 61 vs. 45 msc 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.

483 Effects of Artemisia herba-alba Asso. aerial parts on high-fat diet induced obesity in rats. Y M Rabeeb, A Safaia, M Aouichri, H Laouer, MC Ben Rayana, A Haddii, M Ilatiri. Tunis - Tunisia

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 Artemisia herba-alba in obesity.

In this study, we investigated Artemisia hole aerial parts bioactivity on obesity induced by high-fat diet. The aim of present study was to investigate the potential effect of Artemisia herba-alba Asso (AHA) on high-fat diet induced obesity.

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% (3.5 g/kg/day) safe diet dose of Artemisia’s aerial parts. The control group receive 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 determine glucose, urea, creatinine and lipids plasma levels. Dietary supplementation of Artemisia decreases significantly overweight induced by high-fat diet (48%; P < 0.001). Furthermore, treatment reduces significantly 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, was 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 LDL fraction with Artemisia-fed rats (13%; P < 0.05). Otherwise, treatment reduces efficiently hypercorticismemia 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.


Introduction: Pregnancy consists in complexes physiological processes including immunological and hormonal events with metabolic impacts. Because the evolution of pregnancy is such as 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 plasma 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 cholesterol fall is reported in pregnancy whereas TG decrease during the gestation and rise before the parturition. A reduction of VLDL + LDL in pregnant is noted comparatively to control (13.2 ± 9.27 mg/dl vs. 6.3 ± 1.73 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). A red coloration show a meaningful aggregation of lipids rich cells to vascular endothelium, but few lipid reserves are observed between slices in the aortae. In the liver. TG concentration rises and cholesterol level decreases (P < 0.01).

Conclusion: It seems that pregnancy reduces lipid stores in the local rabbit liver and rises lipid capture and stored in the aorta. Pregnancy hypertiroidism registered in Arabian domestic rabbit seems no pathogenic, this physiological state normalizes after the parturition.
98 Effect of nitric oxide inhibition on glycogen phosphorylase and hexokinase activities in rat liver after water or food deprivation

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 infused livers from fed rats, either no effect, inhibition or stimulation of glycolysis 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 or 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, the glycogen phosphorylase and hexokinase activities were determined in 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: This study proves that nitric oxide may be involved in the regulation of glucose metabolism by modulating the activities of hepatic enzymes of glycolysis and glycogenesis according to physiological conditions.

986 Cortical plasticity of swallowing oral muscle induced by ventilation or swallowing tasks
E Verin*, S Gallas*, JP Marie*, P Denis* "Rouen - France"

Introduction: In stroke patients swallowing rehabilitation is the main treatment of swallowing disorder and it has never been demonstrated which 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 (nTMS) and focal stimulation (fTMS) of M. mylohyoideus were performed, permitting to measure MHEPs and modification in pharyngeal pressure. Thresholds were also measured during nTMS and fTMS. During focal stimulation, cortical area was determined for the right MI muscle (number of point spaced by 2 cm able to product MHEP). During nTMS, MHEP 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 trial, the activities were evaluated.

Results: Swallowing tasks increased the cortical representation of MII muscles, as ventilation did not [Swallowing trial: n = 13 ± 7 points (P = 0.05), Ventilation trial: 1 ± 1 point (P = 0.50)], the amplitudes of the MHEPs and the amplitudes of the MHEPs and the amplitudes of the pharyngeal pressure induced by nTMS increased after ventilation movements and not after swallowing tasks. They were highest during swallowing tasks.

Conclusion: Swallowing task and swallowing 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.

987 Activity of adiponectin, leptin and cytokines in diabetic mothers and their macroscopic offspring

Introduction: It is now well established that intrauterine exposure to sickness during gestation may result in the development of atherosclerosis and cardiovascular disease in later life. Obesity is a major risk factor for cardiovascular disease. It is also well established that children of mothers with obesity during pregnancy have a higher prevalence of obesity and type 2 diabetes than children of normal weight mothers. The objectives of this study were to investigate the role of adiponectin, leptin and cytokines in development of macrosomia in diabetic mothers.

Methods: Serum samples were collected from all patients and the samples were separated. The samples were assayed for adiponectin, leptin and cytokines levels. The results were compared with the control group.

Results: The levels of adiponectin were increased in diabetic mothers compared to control women. The levels of leptin were decreased 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 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.

Conclusion: The results of this study suggest that adiponectin may be a protective factor against macrosomia in diabetic mothers. Leptin may play a role in the development of macrosomia in diabetic mothers. Cytokines may have a role in the development of macrosomia in diabetic mothers.

988 Effect of subchronic exposure to organophosphorus compound on glucose metabolism in rats in vivo
R Reng*, B Mornagui*, A Kamoun*, S El-Fazaa*, N Gharbi*, Tunis–Tunisia 80 Lyc–France

Introduction: The use of pesticides in public health and agricultural programs has resulted in pollution of water, air, and food that include severe acute and chronic cases of human poisonings. In addition, the use of organophosphorus pesticides has led to considerable disruption of the chemistry and durability as compared to organochlorine pesticides. Organophosphorus compounds are primarily recognized for their ability to induce toxicity in mammals through inhibition of acetylcholinesterase leading to accumulation of acetylcholine and subsequent activation of cholinergic muscarinic and nicotinic receptors. The aminopyrine and phenobarbital metabolic tests were used in the present investigation to test the effects of the organophosphorus compound on glucose metabolism.

Methods: Malathion was administered intragastrically by stomach tube in the form of a 1 ml corn oil containing 100 mg/kg body weight for 12 days. At the end of the period, the experiment was concluded. The activities of glycogen phosphorylase and hexokinase were measured in liver homogenates. The methodology employed was a non denaturing electrophoresis followed by activity-staining (native PAGE).

Results: Malathion decreases glycogen phosphorylase activity by 50% and increases hexokinase activity by 15% in liver homogenates. The methodology employed was a non denaturing electrophoresis followed by activity-staining (native PAGE).

Conclusion: These findings were in favour of an activation of glycogen storage by malathion, an organophosphorus insecticide, on hepatic enzymes of glycolysis and glycogenesis in rats in vivo.
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 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). A group of 8 healthy lean and six obese males subjects performed a 45 min exercise bout at 50% of their maximal oxygen uptake either with or without saturated FA. Then, a 2-hours treatment on isolated fat cells, the effect of 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 (phenolamine). Dialysate glycerol concentration was taken as an index of lipolysis.

**Methods:** We tested first *in vitro* on isolated fat cells, the effect of 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 (phenolamine). 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 alpha2-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 alpha2-adrenergic antilipolytic effect was more pronounced in obese than in lean subjects. HFM totally suppressed the alpha2-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 alpha2-adrenergic mediated antilipolysis in adipose tissue. This is another mechanism whereby fat-rich food may contribute to dysregulation of lipid metabolism.

**Methods:** Three hundred students aged of 15–20 years and coming from different zones (rural 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, benefiting 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. The present result demonstrates that saturated FA per se, in *vitro* as well as in *vivo* suppresses alpha2-adrenergic mediated antilipolysis in adipose tissue.

**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 devoted food behaviour, benefitted from the media advertisement, and the consumption 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.

**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:** The 492 short-term exposition to saturated fatty acids inhibits adrenergic alpha2-antilipolytic effect in human adipose tissue.

**Results:** Short-term exposition to saturated fatty acids inhibits adrenergic alpha2-antilipolytic effect in human adipose tissue.

**Conclusion:** Short-term exposition to saturated fatty acids inhibits adrenergic alpha2-antilipolytic effect in human adipose tissue.