Fundamental & Clinical Pharmacology

PHARMACOLOGIE CLINIQUE, MÉTHODOLOGIE

CO-001

What impact of recent recommendations for crushing drugs for elderly patients?

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Crushing drugs is very common in geriatric units. In 2009, a first study, performed in all the geriatric units of a universitary hospital, showed that numerous errors were made during prescription, preparation, administration. The aim of this second prospective study was to assess the impact of regional and national recommendations in the same geriatric units.

A survey was performed in June 2013, including 719 inpatients (85 ± 7 years). For every patient who received crushed drugs, we recorded why drugs were crushed, pharmacological classes, galenic presentations and the technique used for preparation and administration. Results were compared to the previous study. The number of patients receiving drugs after crushing was significantly lower than in the previous study (22.9% vs. 32.3%, $P < 0.001$). The number of crushed drugs was lower too (594 for 165 patients vs. 966 for 221 patients, $P < 0.01$). Indications for crushing drugs remained swallowing disorders (81.8% vs. 78.3%) and psycho-behavioural disorders (19.4% vs. 32.1%). The number of drugs which should not be crushed (orodispersible 38%, prolonged-release 9%, gastro-resistant forms 7%, but mainly in absence of pharmacological study 44%) was lower (24.9% vs. 42.0%, $P < 0.001$). The vehicle was more often neutral (water 88.5% vs. 5.7%, $P < 0.001$). But nurses again crushed drugs together (90.9%). Use of a mortar was less frequent (38.6% vs. 92.6%, $P < 0.001$), in favor of individual specific cups (56.1%). Mortars were more often cleaned between each patient (56.0% vs. 11.6%).

This second study shows that regional and national recommendations led to less crushed drugs with an improved practice.

Technical ameliorations are still possible, but will remain imperfect because lack of therapeutic alternative in some cases and absence of appropriate pharmacological study [1–3].

References:

1. Médicaments écrasés: une pratique 'artisanale' fréquente chez les personnes âgées mais avec un risque iatrogène potentiel. 60ième Congrès de la Société Nationale Française de Médecine interne, Toulouse, 9–12 décembre 2009. Rev Med Int 2009; 30(Suppl 4):S346.

2. L'écrasement des médicaments en gériatrie: une pratique 'artisanale' avec de fréquentes erreurs qui nécessitait des recommandations. Rev Méd Int 2012 (33): 546–551

3. Outils de sécurisation et d'auto-évaluation de l'administration des médicaments (HAS, juillet 2011)

Keywords: geriatrics, crushed drugs, swallowing disorders.

CO-002

Solid oral dosage forms in paediatric patients: a cost-saving investigation

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Background: Oral liquid drugs, considered as the most appropriate oral medicine in children, present, however, numerous disadvantages. Alternatively, solid oral forms have greater stability, provide higher drug compliance in children and markedly reduce costs. Two limits could explain the difficulties of solid drug use in children: children difficulties to swallow and solid dosage low flexibility.

Purpose: Our objective was to assess the substitution suitability of prescribed oral liquid medicines with solid forms for children over 2 years. Cost-savings that could be made if liquid medicines were substituted with an acceptable solid form were determined for NHS prices.

Materials and methods: Substitution suitability for dispensed liquid medicines during 1 week was determined (i) screening for the existence of a marketed solid oral alternative then (ii) evaluating the acceptability of solid forms, firstly related to the posology and secondly to pill size depending on children's age (EMEA guidelines [1]). Treatment cost were calculated on the basis of providing treatment for 28 days for long term treatment and prescribed duration for short term treatment.

Results: Among the 476 liquid medicines dispensed, 90% were available as a marketed solid form. Considering solid form dosage acceptability, 80% of liquid medicines could be substituted with solid form. Only 41% liquid formulations could be substituted when additionally considering pill size.

Drug cost-saving that could follow the substitution of liquid medicine with an acceptable solid form for dosage and size would be £4951 and £8550 respectively for hospital and community, corresponding to an estimate projected annual saving of £238 and £410 K.

Conclusion: Surprisingly, almost all liquid medicines were available in an acceptable dosage. Whilst not all children over 2 years will be able to swallow tablets, this study has shown the importance of potential drug cost savings if solid forms were used in children and may provide a theoretical basis for modification of healthcare behaviours.

Reference:

1. The Committee for Medicinal Products for Human Use (CHMP). Draft Guideline on Pharmaceutical Development of Medicines for Paediatric Use,2011. EMEA/CHMP/QWP/180157/2011.

Keywords: drug formulation, paediatric, swallowing, oral drug delivery.

CO-003

Pharmacological and clinical factors influencing the quality of life profiles of renal transplant recipients

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Objective: Health quality of life (HQOL) is a way to measure the impact of the renal transplantation on the global well-being of the transplant recipients. The SF-36 survey is one of the most widely used. The aim of the present study was to investigate factors influencing the SF-36 mental composite score (MCS) HQOL over the time in renal transplant recipients.

Methods: In the EPIGREN cohort, repeated HQOL evaluations using the SF-36 survey, adverse drug effects and clinical follow-up data were collected in 297 renal transplant recipients, within 36 months post-transplantation. K-means for longitudinal data was used to identify clusters of time-profiles of SF-36 MCS over the first 36 months post-transplantation. To determine association between MCS QOL and covariates, random forests were used. Statistical analysis was carried out with the R software (Version 2.15.2) using KML and rpart packages. Results were considered as significant if $P < 0.05$.

Results: Cluster analysis found two clusters for the time course of MCS. The first cluster, with a low score, was composed of 119 (40%) trajectories (i.e. 119 patients), with an average MCS of 38.5 (95% CI: 37.4–39.6). In this cluster, the mean trajectory of MCS was significantly lower than both the MCS score reported in dialysis population (MCS: 40.7) and the score reported in the general population (MCS: 47.2). The second cluster, with a good MCS, was composed of 178 (60%) trajectories, with an average MCS of 50.1 (95% CI: 49.6–50.5) significantly higher than the score reported in the general population. Probability to belong to the low MCS cluster increase with (i) serious infection (viral, bacterial and fungal) reported by the physician (OR = 2.83), (ii) muscle weakness and anxiety reported by patient only, (iii) length of hospitalisation stay in the fist year post-transplantation, and decrease with increase of mental and physical HQOL at 1 month (OR = 0.9).

Discussion: Regarding the mental composite score, two subpopulations were identified together with factors influencing the HQOL (Adverse effects, both mental and physical HQOL in the first month…). The analysis of all dimension of SF36 must complete this result to guide clinical management [1].

Reference:

1. Rapport qualité de vie – REIN. http://www.invs.sante.fr/publications/2008/insuffisance_renale/rapport_insuffisance_renale.pdf

Keywords: renal transplantation, quality of life, clusters.

CO-004

Hospitalisation for ovarian hyperstimulation syndrome: prevalence in a mono centric cohort study

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Objective: We propose to evaluate the risk of hospitalisation for ovarian hyperstimulation syndrome (OHSS) in patients undergoing induction of ovulation for in vitro fertilization (IVF).

Material: The present study involves 586 patients who benefited IVF in a French Center with ovarian stimulation cyle occurring in 2012.

Method: It was an observational study recording all the cases of hospitalisation for OHSS. The database was obtained from the medical record. The declaration was made simultaneously to the pharmacovigilance center and to the Agency of the Biomedecine. Each patient was monitored at least 5 weeks after treatment, more if pregnancy occurred. In case of hospitalisation the report was taken into account. Descriptive analysis of the population was performed and Khi2 or t test analysis used to compare hospitalised for OHSS and non hospitalised women.

Results: There were 738 cycles with ovarian follicles pick up, 9 patients (1.2% of cycles) were hospitalised for OHSS. These patients were younger ($P < 0.001$) and thinner ($P < 0.0001$) than others. They have more frequently received a recombinant FSH than urinary FSH ($P < 0.03$). The protocol used (short, long, agonist, antagonist) did not influence hospitalisation rate. The cycle length and the number of follicles were more important in hospitalised cases but there is no significant difference. The number of transfer and the mean number of embryo transferred were less important but not significantly different. The rate of pregnancy after transfer was greater but not significantly different. Two patients were hospitalised for pleural effusion, 5 for ascitis and 2 for elevated Estradiol or number of oocyte recovered. The mean duration of hospitalisation was 9.3 d ± 3. There was no other complication such as thrombosis. In this group, two patients were at risk of HSSO, one for history of uncontrolled response, the other for polycystic ovary syndrome.

Discussion: OHSS is the second complication of hormonal IVF treatment. Patient screening, use of short protocol for patient at risk and postponed embryo transfer allowed to reduce hospitalisation to cases in which OHSS was unexpected [1].

Reference:

1. Asch RH and coll. Severe ovarian hyperstimulation syndrome in assisted

reproductive technology:definition of high risk groups. Hum Reprod 1991;6 (10):1395–9.
Keywords: observational study, pharmacovigilance, ovarian hyperstimulation, assisted reproductive technology.

CO-005
Memantine, *N*-Methyl-D-Aspartate (NMDA) receptor antagonist, a promising preventive drug for post-mastectomy pain
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Aim of investigation: NMDA receptor antagonists are potential therapies for neuropathic pain. Clinical trials show contradictory results as regard neuropathic pain alleviation and side-effects at therapeutic doses may limit their clinical use. Memantine, prescribed in Alzheimer's disease to improve cognitive function has minimal side-effects and could be an interesting drug to prevent or treat neuropathic pain. Considering the high prevalence of breast cancer and of post-mastectomy neuropathic pain, a clinical trial has been carried out to evaluate if memantine administered before and after mastectomy prevents pain development and maintains cognitive function and quality of life compared to the placebo group.
Methods: A memantine/placebo-controlled, randomized, simple-blind clinical trial (NCT01536314) included 40 women undergoing mastectomy for breast cancer at the anticancer Hospital, Clermont-Ferrand. Memantine (5–20 mg/day; $n = 20$) or placebo ($n = 20$) was given for 4 weeks starting 2 weeks before surgery. Intensity of pain, cognitive function, quality of life and of sleep, anxiety and depression have been evaluated by different specific questionnaires. Primary endpoint was the pain intensity evaluation by numerical scale (NS) between memantine and placebo groups at 3 months post-mastectomy. The pain intensity differences of both treatments were compared by Student t test ($P < 0.05$ significance).
Results: With memantine, at 3 months, pain was significantly diminished (ΔNS: placebo: 0.7 ± 1.8; mémantine: -1.0 ± 2.0; $P = 0.011$), neuropathic pain induced by chemotherapy was significantly reduced (ΔDN4 chemotherapy, placebo: -0.5 ± 0.8; memantine: -2.1 ± 1.6; $P = 0.011$) and affective component of McGill Pain Questionnaire was improved (placebo: 10.0 ± 13.2; memantine: 1.4 ± 1.9; $P = 0.029$). No significant difference was obtained on cognitive function, quality of life, sleep, anxiety and depression.
Conclusions: Memantine, when administered before mastectomy, prevents 1- painful symptoms induced by mastectomy and 2- neuropathic pain symptoms induced by chemotherapy. This promising strategy constitutes a major progress in the management of post-operative and chemotherapy-induced pain.
Keywords: memantine, post-mastectomy pain, neuropathic pain, chemotherapy.

CO-006
Optimization of single dose post-operative nefopam therapy in the elderly on the basis of pharmacokinetics and pharmacodynamics
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Aims: Nefopam is a non-morphinic central analgesic for which no recommendation exists concerning adaptation of regimen in aged patient with or without renal impairment. The objective was to describe the pharmacology of nefopam in aged patient with or without renal impairment, to develop guidelines for practical use.
Methods: Elderly patients ($n = 48$), 65–99 years, with severe, moderate renal-impairment or with normal renal function, were recruited. Nefopam (20 mg) was administered as a 30-min infusion post-operatively. Pharmacokinetic parameters of nefopam and desmethyl-nefopam were derived for each patient by using the empirical-bayes-estimates of the final validated population pharmacokinetic model. Values (i.e. cut-values) of predictors, as pharmacokinetic parameters, corresponding to a probability higher than 0.6 for side effects development and to a probability lower than 0.5 for morphine requirement were determined using multivariate logistic regression analysis. The regimens needed to achieve these targets were studied by monte carlo simulations using Monolix (V 4.1) software.
Results: Morphine requirement was related to nefopam exposure (OR = 0.987 for every 1 µg/L/h, $P = 0.02$) with a cut-value of 947 µg/L/h. Tachycardia and postoperative nausea and vomiting (PONV) were best associated with maximal concentration (Cmaxnef) of nefopam (at least OR = 1.58 for every 50 µg/L, $P = 0.02$) and the rate of increase (RCmaxnef) in nefopam plasma concentration (at least OR = 1.26 for every 50 µg/L/h, $P = 0.02$) with respectively a cut-value of 437 µg/L and 859 µg/L/h. The probability to observe nefopam exposure higher than values linked to morphine requirement was around 0.70 whatever the duration of nefopam infusion (15, 30, 45, 60-min). The probabilities to observe C_{maxnef} and RC_{maxnef} lower than values linked to development of tachycardia or PONV was at least equal to 0.95 for a duration of infusion higher than 45-min.
Conclusions: We identified nefopam pharmacokinetic predictors for morphine requirement and side effects such as tachycardia and PONV. In order to maintain morphine sparing and decrease side effects following a single dose of nefopam (20 mg), simulations suggest an infusion time of at least 45 min in the elderly with or without renal impairment.
Keywords: analgesia, elderly, exposure-response, logistic regression, nefopam, population pharmacokinetics, renal impairment.

CO-007
Use of multi-state model in pharmacoepidemiology: illustration with methadone
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Objective: In pharmacoepidemiology studies, survival analysis methods are frequently applied to investigate use of medications. However in real settings, multiple transitions may occur before the end of follow-up. The best way to model them is to use multi-state models (MSM) also called Markov models. MSM can be defined as a generalization of the survival analysis in which intermediate states are identified. The aim of this study was to describe, using MSM, the transitions by methadone users between the two formulations (syrup and capsule which is available since 2008).
Methods: This study is based on reimbursement database in Provence-Alpes-Côte d'Azur Corse region. Incident methadone users (at least two reimbursements of methadone between February 1st, 2010 and November 30th, 2011) from this region affiliated to the French health reimbursement general system have been included. Follow-up of patients was done every month from their inclusion. Four states were defined for each month of follow-up: syrup (delivery of syrup form only), capsule (delivery of capsule form only), syrup-capsule (delivery of both forms) and non user (no methadone delivery). The model was fitted using R 3.0.2 software and the *msm* package version 1.2.
Results: 1 265 methadone incident users were included (38% of all methadone users in 2010–2011) and 16 404 transitions occurred. When patients were in syrup-capsule state, they had 51% of chance to move to capsule and 19% to move to syrup. The probability of moving from syrup state to non user state was 13% (10% from capsule state to non user state). The average length of stay (during the 23 month observation period) was 6.7 months (CI 95%: [6.2–7.3]) in capsule state, 5.1 (CI 95%: [4.9–5.5]) for syrup state, 6.3 (CI 95%: [5.9–6.8]) for non user state and 0.53 (CI 95%: [0.47–0.58]) for syrup-capsule state. The model provided good predictions of prevalence for each state over 23 months.
Conclusion: This study confirms that capsule form lead patients to stay with it more than syrup form. Besides, it shows the utility of MSM for modeling multiple sequences of drugs use in pharmacoepidemiology.
Keywords: multi-state model, markov model, pharmacoepidemiology, methadone, galenic formulation.

CO-008
Sources of disagreement between investigator and sponsor in causality assessment of serious adverse events during academic clinical trials
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Objective: To describe sources of disagreement between investigators and the sponsor (Bordeaux teaching hospitals) in causality assessment of serious adverse events (SAEs) during academic clinical trials (CTs).
Methods: All SAEs reported in year 2011 during academic CTs, with disagreement in causality assessment, were included in the study. For each SAE, causes of disagreement were reviewed by an event validation committee (two experts of pharmacology and clinical trial safety and vigilance).
Results: 48 SAEs with disagreement in causality assessment were identified in 2011. For 32 SAEs (67%), the sponsor presented arguments for the assessment: 10 SAEs (21%) were possibly related to the research according to investigator, but sponsor excluded a causal relationship since patients were in the control group; for 20 SAEs (42%) the sponsor envisaged a causal link based on chronologic and semiologic criteria, while the investigator retained another cause (6 SAEs, 13%) or did not specify any other cause (14 SAEs, 29%); the investigator did not provide a causality assessment for 2 SAEs (4%) considered as not related to research by the sponsor face to chronologic and semiologic criteria. For 16 SAEs (33%), the sponsor retained a causal relationship with the research as it could not be excluded, while the investigator retained another cause for 9 SAEs (19%) or did not specify any other cause (7 SAEs, 14%).
Discussion: CT regulations require that investigator and sponsor assess whether there is a reasonable possibility of a causal relationship between the research and the occurrence of SAE. Part of discrepancies may be explained by a lack of knowledge of investigator about CT regulations. In some cases, the investigator seems not to retain the research role when another cause exists and does not take into account a possible concomitant role of study. The sponsor concludes with arguments in most cases, but also retains the responsibility of the research if arguments to exclude it are missing even though the investigator describes another possible cause. A causality assessment method should be built for clinical trials in order to determine criteria and rules of assessment for both investigators and sponsor.
Keywords: academic clinical trials, disagreement, causality assessment.

CO-009
Competing risks: why should we care about in pharmacoepidemiology research?
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Objective: To illustrate how competing risks can modify associations in pharmacoepidemiology studies.
Design, settings, and participants: The first 20 years of follow-up of the PAQUID study on ageing were used to assess the risk of incident dementia

associated to the initiation of benzodiazepines in subjects aged 70 and over [1]. The analyses used mutivariable Cox proportional hazard models. Before manuscript acceptation, referees pointed out a possible competing risk of death in elderly on the study results. As this risk remains poorly considered in pharmacoepidemiology, we decided to perform simulations to illustrate the potential effect of competing risks on association estimates. Competing event is an outcome that occurs frequently in the studied population and may interfere with other risk evaluations. Death is classically considered as such in elderly populations. To study the modifications that can be observed for the association between benzodiazepine initiation and the risk of dementia, we simulated variations in the Hazard Ratio of death among benzodiazepine initiators and non-initiators included in our study, the incidence of dementia being unmodified. Analyses were performed using Cox proportional Hazard models similar to that used in the initial study.
Results: In the initial publication, 30 cases of dementia were confirmed in the 95 benzodiazepine initiators, and 223 in the 968 non-initiators. The Hazard Ratio (HR) for the association estimated initially through Cox models was 1.6 (1.1–2.4); HR for death was 1.1 (0.8–1.5) indicating no potentiality of competing bias. When modifying HR for death to 1 and to 0.9 for benzodiazepine initiators, the Cox estimated HR for dementia was 1.4 (0.9–2.0), and 1.1 (0.8–1.7), respectively. When increasing the HR of death to 1.3 and 1.6, these were 1.7 (1.2–2.6), and 2.1 (1.4–3.1), respectively.
Conclusion: When studying a risk in a population in which other events are likely to frequently occur and to impact follow-up duration, competing risks should be considered when estimating associations. In particular, the risk of death should be systematically checked in pharmacoepidemiology studies conducted in the elderly.
Reference:
1. Billioti de Gage S et al. Benzodiazepine use and risk of dementia:prospective population based study. BMJ 2012;345:e6231.
Keywords: pharmacoepidemiology, competing-risk.

PHARMACOLOGIE MOLÉCULAIRE ET PRÉ-CLINIQUE

CO-010

Involvement of IL-1beta and TGF-beta1 in the fibrogenesis process mediated by immortalized human hepatic stellate cells, the LX-2 cell line
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Liver fibrosis is a physiological response due to acute injury and resulting in an inflammatory process, imbalance between matrix metalloproteinases (MMPs) and the tissue inhibitors of metalloproteinases (TIMPs), and the production of scar tissue (collagen). When the injuries persist, excessive collagen deposition could lead to serious pathologies. At cellular level, collagen production is mediated by activated fibroblasts, also known as hepatic stellate cells. This activation occurs after crosstalk between fibroblasts and liver macrophages which express pro-inflammatory cytokine interleukin-1 beta (IL-1β) cleaved into active form by caspase-1 associated with NLRP3-inflammasome pathway. We compared the potential of activation by IL-1β or TGF-β1 on the MMPs/TIMPs imbalance and on the production of collagen in immortalized activated hepatic fibroblasts, the LX-2 cell line.
LX-2 cells pre-treated or not with endogenous interleukin-1 receptor antagonist (IL-1Ra) were treated into proliferative state for 24, 48 or 72 h with recombinant human interleukin-1 beta (rhIL-1β). The cells were also treated with human recombinant transforming growth factor beta 1 (rhTGF-β1) for 24, 48 or 72 h to evaluate the pro-fibrogenic potential of cells. The mRNA expression was evaluated by RT-qPCR and proteins secretion was measured in the supernatant using ELISA method.
rhTGF-β1 (1, 5 or 10 ng/mL) induced an increase in mRNA levels of MMP-2, TIMP-1, α-SMA, collagens type I and IV and a decrease in MMP-1 and MMP-3 mRNA or proteins levels. In contrast, treatments with rhIL-1β (1 or 10 ng/mL) showed increased levels of MMP-1, MMP-3 and MMP-9 but not MMP-2 mRNA or proteins levels and also showed reduced levels of α-SMA, collagens type I and IV, TIMP-2 and TIMP-3 but not TIMP-1 mRNA or proteins levels. These effects were totally or partially reduced by pre-treatment with IL-1Ra.
Taken together, these results demonstrate that LX-2 cells are able to improve their pro-fibrogenic environment when treated with TGF-β1 but that treatments with IL-1β trigger an anti-fibrogenic environment on these cells. This suggests an important role of the MMPs/TIMPs imbalance regulation on the production of collagen for the control of chronic liver fibrosis mediated by macrophages on fibroblasts.
Keywords: fibrogenesis, liver, cytokines, metalloproteinase, stellate cells.

CO-011

Imaging angiogenesis: an innovative radiolabeled RGD-targeting dendrimer
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Angiogenesis is a critical step targeted for therapy purposes, either for being suppressed in tumorogenesis and metastasis, or rather researched for post-ischemic contexts. To date, angiogenesis imaging is a promising tool to evaluate oncologic or ischemic patients and help physician in patient management for stratification and therapy follow-up. Our work aims at delivering a proof of concept of using radiolabeled dendrimersomes for molecular imaging of angiogenesis in an ischemic hindlimb rodent model. No such nanoradiopharmaceutical has been approved so far.
Materials and methods: A bifunctional αvβ3-integrin-targeting dendrimersome was designed, synthesized and radiolabeled with 111-indium chloride. The dendrimersome results from the self-assembly of 2 different dendrimer conjugates: one for imaging ([111In]NOTA-conjugate, 92 MBq/100 μg) and one for targeting (RGD-conjugate), mixed with a 3:1 ratio, under sonication. Previous studies ensured a dendrimersome stability for more than 4 days in serum. Preliminary biodistribution studies were carried out by SPECT/CT imaging: both early dynamic planar acquisitions (T + 0 to T + 20' post-IV injection of 7 MBq, 7.5s frames) and late multi-pinhole SPECT/CT (T + 120', D + 1 and D + 2 or D + 3) were performed using a Bioscan NanoSPECT/CTplus camera, 20 days after ischemia. Mice surgery was carried out in accordance to Helsinki Declaration and underwent the local preclinical ethics committee agreement.
Results: We reached a radiolabeling yield >90% and stability >48 h. We first confirmed the biodistribution patterns between free indium and radiolabeled dendrimersomes. The dendrimersomes were mainly eliminated through the urinary system at an early stage. A liver uptake was visualized 24 h post-injection with both dendrimersomes. More interestingly, with our preliminary preclinical study, we got a [111In]-NOTA-RGD-dendrimersome uptake localized on the ipsilateral ischemic hindlimb (ipsi/controlateral ratio (i/c) = 2.5 ± 0.5 at D + 1), that wasn't obtained with the non specific [111In]-NOTA-PEG conjugate (i/c = 1.1 ± 0.4 at D + 1).
Conclusion: Compared to other nanocarriers, dendrimersome-based delivery has several advantages: high drug payload, stable formulation and controllable structure. With this innovative vector, we managed to target specifically angiogenesis in an ischemic zone. Upcoming steps will consist in validating these preliminary results in tumor models (prostate tumor xenografts). We also plan to target the SSTR2 with a dendrimer conjugate based on an octreotide analog.
Keywords: angiogenesis, RGD, radiolabeling, ischemia, SPECT, dendrimer.

CO-012

Monosodium urate (MSU) induced IL-1beta via a purinergic receptor/NLRP3-inflammasome pathway from macrophages
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Monosodium Urate (MSU) has been described as a danger signal activating NLRP3-inflammasome pathway. We investigated the role of P2 purinergic receptors in the activation of NLRP3-inflammasome pathway after MSU treatment in human macrophages. Monocytes from healthy donors were obtained from buffy coat (EFS, Rennes) using human CD14 Microbeads separation kit. Macrophages were obtained after differentiation from monocytes by incubation with rhGM-CSF for 7 days. P2R transcription was extinguished by electroporating specific siRNA. mRNA expression was evaluated by RT-qPCR and production of proinflammatory cytokines was measured in the supernatant using ELISA. MSU crystals (500 μg/mL) were able to induce the release of IL-1β from human monocyte-derived macrophages induced by low concentration of LPS (0.1 μg/mL) at 6 h. Moreover, caspase-1 inhibitor (z-YVAD-fmk) dose-dependently decreased production of IL-1β after LPS + MSU treatment. Quantitative PCR analysis showed that treatment with LPS and MSU induced a significant increase in the expression of NLRP3 and IL-1β after treatment with LPS alone or associated with MSU at 6 h. Furthermore, we also showed that MSU treatment induced P2X7R mRNA expression at 6 h by RT-qPCR. Electroporation of siRNAs directed against P2 receptor led to an efficient knock-down of P2R mRNA in macrophages at 24 h and 48 h. We showed that siP2X7R, but not siP2X4R, was able to reduce the release of IL-1β from macrophages stimulated by LPS + MSU at 6 h. Moreover we observed that A-740003, a P2X7 purinergic receptor antagonist decreased IL-1β production after treatment with LPS and MSU. Our results show the involvement of purinergic receptor and NLRP3 inflammasome pathway in the secretion of IL-1β from MSU-stimulated human macrophages and suggest that blockade of the NLRP3 inflammasome or $P2X_7$ receptor represents a novel potential therapeutic approach to control inflammation.
Keywords: NLRP3-inflammasome, purinergic receptor, monosodium urate, macrophages, P2X7R.

CO-013

PART 1- Traumatic brain injury by controlled cortical impact in mice – time courses of neuroinflammation, corpus callosum demyelination, sensorimotor deficits, edema and lesion
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Objective: Traumatic brain injury (TBI) results in neuroinflammation that may participate to white matter injury (WMI). The latter, evidenced by demyelination, is associated with neurological disorders. As only few data are available, the objective was to study their time-courses after TBI induced by controlled cortical impact on adult male Swiss mice between 6 h and 12 weeks post-TBI.
Materials: *Neuroinflammation* was determined by microglia activation using CD11b immunostaining (total of $CD11b^+$ cells in a Region Of Interest on 6 brain slices corresponding to brain lesion), *demyelination* by western-blot expression of Myelin Basic Protein (MBP) and Cyclic Nucleoside Phosphodiesterase (CNPase) on *corpus callosum* a cerebral structure enriched in white matter, *sensorimotor and motor coordination deficits* by string, grip and adhesive removal tests, *cerebral edema* using brain water content, and *lesion* by cresyl violet staining.
Results: Microglia activation was present from 24 h (558 ± 51 $CD11b^+$ cells vs. 121 ± 55 $CD11b^+$ cells for sham-operated mice, $P < 0.001$) to 7 days post-TBI (390 ± 42 $CD11b^+$ cells, $P < 0.001$), showing neuroinflammation. TBI induced a decrease of MBP protein expression in the ipsi- and contralateral *corpus callosum* at 7 days (ipsilateral: 1437 ± 289 vs. 3572 ± 536 AU for sham-operated mice, $P < 0.01$; contralateral: 1160 ± 276 vs. 4269 ± 700 AU for sham-operated mice, $P < 0.01$) that persisted at 12 weeks (ipsilateral: 885 ± 459 AU, $P < 0.01$; contralateral: 1141 ± 476 AU, $P < 0.01$), demonstrating bilateral demyelination. Moreover, it promoted a bilateral increase of CNPase expression at 48 h (ipsilateral: 273 ± 41 vs. 99 ± 29 AU for sham-operated mice, $P < 0.01$; contralateral: 238 ± 42 vs. 118 ± 28 AU for sham-operated mice, $P < 0.01$) suggesting TBI-induced maturation of non-myelinating to myelinating

oligodendrocyte with a decrease at 12 weeks post-injury. Sensorimotor deficits were observed at 6 h until 72 h and at 12 weeks. TBI increased brain water content at 6 h (81.6 ± 0.2% vs. 80.2 ± 0.2% for sham-operated mice, $P < 0.001$) that persisted until 5 days (81.4 ± 0.4%, $P < 0.01$), demonstrating brain edema. It promoted a cerebral lesion at 24 h (9.4 ± 2.5 mm^3 vs. 0.4 ± 0.3 mm^3 for sham-operated mice, $P < 0.001$) persisting up to 72 h (5.6 ± 1.8 mm^3, $P < 0.01$) that evolved toward a scar.

Conclusion: Our data give an overall view of neuroinflammation, demyelination and sensorimotor deficits in experimental TBI that could help to validate pharmacological strategy for preventing post-traumatic WMI. As demyelination is associated to neurobehavioral disorders, this was examined and presented in another abstract submitted to P2T2014.

Keywords: traumatic brain injury, neuroinflammation, demyelination, neurological deficits.

CO-014

EMMPRIN/CD147 is a co-receptor for VEGFR-2 in tumor angiogenesis: towards its inhibition

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Tumor-stroma interactions promote malignancy by modifying tumor microenvironment. EMMPRIN (CD147), a membrane glycoprotein enriched on the surface of tumor cells, is an important mediator of tumor-stroma cooperation. We have previously shown that EMMPRIN can: 1) induce matrix proteases (MMPs, uPA) in stromal cells; and 2) promote angiogenesis by regulating VEGFR-2 via HIF-2a in endothelial cells. These functions of EMMPRIN were also observed in tumor cells with consequences on their malignant properties, suggesting that they represent a more general mechanism.

As VEGFR-2 has been implicated in regulation of cell migration, proliferation and apoptosis, we looked for a potential role of EMMPRIN in the activation of this receptor.

Our results point to a unique mechanism of action of EMMPRIN involving the activation of VEGFR-2 tyrosine kinase receptor independently of its ligand, through a direct interaction with EMMPRIN. The domain of EMMPRIN involved in VEGFR-2 interaction and activation was identified by functional domain analysis of EMMPRIN conducted by cloning and expressing its different domains. Computational docking analyses followed by site-directed mutagenesis allowed us to predict and characterize the nature of this interaction. Our results suggest that EMMPRIN is a new co-receptor for VEGFR-2 and is therefore a promising target for blocking tumor-related angiogenesis. Our main goal is to develop specific pharmacological inhibitors that are able to block this interaction for use in preclinical and clinical stages.

Keywords: tumor angiogenesis, EMMPRIN/CD147, VEGFR-2, targeted therapy.

CO-015

Imidazoline I1 receptor ligands activate hepatic adiponectin pathways and thus improve insulin sensitivity

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Introduction: Metabolic syndrome is defined as a cluster of interrelated risk factors, which promotes the development of cardiovascular and metabolic diseases. Previous studies in rat models of metabolic syndrome have demonstrated that ligands selective for I1 imidazoline receptor (LNPs) increase insulin sensitivity through central sympathoinhibitory action and an additional peripheral effect. A part of this peripheral beneficial effect could be attributed to adiponectin, a major insulin-sensitizer adipokine. The objective of this study was to explore the possible direct actions on hepatocytes, one of the target cells of insulin and adiponectin.

Methods: Experiments were carried out in HepG2 cells, a cell line of hepatocytes. In order to evaluate the effect of LNPs on insulin sensitivity, the activation (i.e. phosphorylation) of a key actor of insulin pathways, AKT, was evaluated by measuring the ratio pAKT/AKT by Western Blot. Similarly, the effect of LNPs on adiponectin signaling was evaluated by measuring the rate of phosphorylation of the central kinase involved in adiponectin pathways, AMPK, by Western Blot.

Results: Insulin (10 μM) induced a marked phosphorylation of AKT (pAKT/AKT = 0.49 ± 0.16) compared to control without insulin (pAKT/AKT = 0.11 ± 0.03; $P \leq 0.05$) whereas LNPs (1 μM) alone did not increase AKT phosphorylation. Interestingly, pretreatment by LNPs (1 μM) during 60 min could potentiate the insulin-induced activation of AKT: LNP509: pAKT/AKT = 1.13 ± 0.18 ($P \leq 0.05$ vs. insulin alone); LNP599: pAKT/AKT = 1.23 ± 0.16 ($P = 0.0545$ vs. insulin alone).

Concerning adiponectin signaling pathways, LNPs alone (from 10^{-9} M to 10^{-4} M) increased AMPK phosphorylation in a concentration- and time-dependent manner. The maximal effect was obtained after 10 min exposure of LNPs 10 μM (untreated cells: pAMPK/AMPK = 0.18 ± 0.04; LNP 509 pAMPK/AMPK = 0.38 ± 0.05 $P \leq 0.05$; LNP599 pAMPK/AMPK = 0.46 ± 0.17).

Conclusion: These data suggest that LNPs on hepatic cells activate adiponectin pathways and potentiate insulin action. These two direct effects on insulin sensitive cells could account for the ameliorated insulin sensitivity observed in vivo.

Keywords: adiponectin, imidazoline-like drugs, insulin sensitivity, metabolic syndrome.

CO-016

Specific brain activation patterns of serotonin-1A agonists detected by pharmacological-MRI

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Introduction: Serotonin 1a (5-HT_{1A}) receptors constitute an important therapeutic target for neuropsychopharmacology. The recent concept of 'biased agonism' asserts that highly specific agonists can preferentially direct receptor signaling to specific intracellular responses. This opens the possibility of drugs targeting receptor in specific brain regions. Our aim is to bring supplementary proofs to this concept with in vivo imaging. Functional magnetic resonance imaging (fMRI) with the blood oxygen level dependent (BOLD) signal has recently been employed to investigate the effects of psychoactive agents on brain activity (pharmacological-MRI).

Material and method: The present study aims to highlight the BOLD signal obtained subsequently to the administration of 5-HT_{1A} agonists. We used three different 5-HT_{1A} agonists (8-OH-DPAT, F13714 and F15599) and one 5-HT_{1A} antagonist (MPPF) to test for their influence on the brain BOLD signal in anaesthetized rats. The in vivo MRI experiments were conducted on a 7T BRUKER Biospec scanner using a T2* EPI sequence. Each animal was scanned first after NaCl ip injection and, 72 h later, after injection of one of the three agonists: 8-OH-DPAT (0.32 mg/kg ip, $n = 8$), F15599 (0.16 mg/kg ip, $n = 6$) or F13714 (0.04 mg/kg ip, $n = 9$) or the antagonist (MPPF, 0.16 mg/kg ip, $n = 6$). The agonist doses were chosen according to previous in vivo studies and imaging data were analyzed using SPM (statistical parametric mapping), a statistical technique used in functional imaging data.

Results: MPPF administration didn't modify the brain activity, confirming its 'silent antagonist' property. The agonist injections led to significant modifications of the BOLD patterns in the whole brain according to the 5-HT_{1A} agonist. If 8-OH-DPAT injection prompted a BOLD effect propagating to large cortical and subcortical regions, F15599 and F13714 stimulated only specific brain regions (e.g. cingulate cortex for F1559 and hippocampal commissure for F13714), confirming their preferential activation of G-protein subtypes.

Discussion: Our study revealed for the first time specific BOLD signal patterns of biased agonists in comparison to a classical agonist. Pharmacological-MRI, transferable to clinic, might be useful for in vivo mapping of the neuronal activation via new 5-HT_{1A} agonists, opening a new way in the screening of drug candidates.

Keywords: pharmacological-MRI, serotonin, 5-HT1A receptor, biased agonist.

CO-017

A functional tandem between transient receptor potential canonical channels 6 (TRPC6) and calcium-dependent chloride channels in human epithelial cells

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TRPC6 plays important physiological functions in the organism and is implicated in pulmonary hypertension, cardiovascular diseases, focal segmental glomerulosclerosis and seems to have a role in cancer development. Until now the pharmacology of TRPC6 remained poorly developed. Guanabenz, an α2-adrenergic agonist used for hypertension treatment (Wytensin®), is an activator of calcium-dependent chloride channels (CaCC) in human Cystic Fibrosis (CF) nasal epithelial cells by transiently increasing $[Ca^{2+}]i$ via an influx of extracellular Ca^{2+} [1].

By single cell fluorescence imaging, we show that guanabenz is an activator of CaCC in freshly dissociated human bronchial epithelial cells from three CF patients with various genotypes (F508del/F508del, F508del/R106C, F508del/H1085R). Using iodide efflux assay and single cell fluorescence imaging we further characterised the effect of guanabenz and show that it is independent of α-adrenergic receptors, is inhibited by the TRPC family inhibitor SKF-96365 but not by the TRPV family inhibitor ruthenium red. Using western-blotting, Ca^{2+} measurements and assays to measure chloride channel activity, we found that TRPC1 siRNA has no effect on guanabenz induced responses whereas TRPC6 siRNA prevented the guanabenz-dependent Ca^{2+} influx and the CaCC-dependent activity stimulated by guanabenz.

Here we show that TRPC6 channel is pivotal for the activation of CaCC by guanabenz through a α2-adrenergic-independent pathway in human epithelial cells. This coupling between two different ion channels is reminiscent of TRPC6 and BKCa channels interactions in podocytes [2, 3]. We propose guanabenz as a new pharmacological tool for exploring TRPC6 and CaCC channels functions and corresponding channelopathies.

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Keywords: calcium-activated chloride channel, canonical transient receptor potential channel, cystic fibrosis, human epithelial cells.

CO-018

Uric acid induced the release of IL-6 and IL-8, but not of IL-1beta and expression of inflammasome pathway in human keratinocytes

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The Keratinocytes/fibroblasts 'cross-talk' is known to play an important role in the inflammatory process, wound healing and homeostasis of the extracellular matrix (ECM). In this study, we investigated the role of inflammatory process in human keratinocytes *in vitro*.

The release of pro-inflammatory cytokines (IL-1β, IL-6, IL-8) and mRNA expression were analyzed by ELISA and qPCR respectively, following incubation of

primary (HEK) and immortalized (HaCaT) keratinocytes with either lipopolysaccharide (LPS, 1 μg/mL) or Uric Acid (monosodium urate, MSU, 1 mg/mL) or the allergen Dermatophagoides farinae (Der-f-1, 10 μg/mL). The expression of different inflammasome components was investigated: Nod-like receptors (NLRP1, NLRP2, NLRP3, NLRP6, NLRC4, AIM2), toll-like receptors (TLR 1–10) and purinergic receptors (PX1-7 and PY2, 6, 11–13)). We also used a phospho-kinase array kit for and western blot technique for the analysis of the phosphorylation pathways.
Pro-IL-1β mRNA expression was enhanced following the stimulation with MSU and Der-f-1, but no difference was observed on the IL-1β production by ELISA. In contrast, IL-8 was significantly released in the presence of MSU and Der f 1 and IL-8 mRNA expression was increased. Barely detectable mRNA levels of TLR4, ASC and NLRP3 were observed suggesting that NLRP3-inflammasome pathway is not involved. NLRP(1-2), AIM2, P2X(4-5-6-7) and P2Y(2-6) were also expressed but any induction by MSU was noted. Phosphokinase array kit revealed an activation of P53, WNK1, ERK1/2 and P38α signaling pathway which was confirmed using western blot technique.
In conclusion, we observed that (HaCaT) keratinocytes express Pro-IL-1β but do not process it into an active form via the caspase 1-NLRP3 inflammasome dependent pathway. MSU are able to elicit an *in vitro* inflammatory process by different pathways such as ERK1/2 leading to the increase in mRNA expression and release of IL-6 and IL-8. Further studied will be focused on the studies of different pathways involved in the expression of IL-8 in both normal an immortalized keratinocytes.
Keywords: keratinocytes, inflammasome, extracellular matrix, wound healing, skin homeostasis.

COMMUNICATIONS EN PHARMACOLOGIE

CO-019

Early and delayed IL-1 beta antibody gevokizumab treatments prevent cardiac remodeling and reverse coronary endothelial dysfunction following myocardial infarction injury in Goto Kakizaki rats

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Aims: Cardiac interleukin-1 beta (IL1-β) production is enhanced acutely after myocardial infarction and is involved in myocardial damages. We tested if early and delayed IL1-β modulations by IL-1β antibody, gevokizumab, prevent left ventricular (LV) remodeling and endothelial dysfunction induced by LV ischemia/reperfusion (I/R) in diabetic rats.
Methods: Gevokizumab (Gevo; 10 mg/kg) was administered 1 h (early) or 7 days (delayed) following reperfusion, after a 20 min of transient ischemia induced by LV artery occlusion and continued every week for 90 days. Delayed perindopril (1 mg/kg) was used as a positive control. LV remodeling and function were assessed (Echocardiography) at 7 and 90 days. LV hemodynamics (Millar catheterization) and relaxation of isolated coronary arteries to acetylcholine (Mulvany wiregraph) were evaluated at 90 days. Collagen density and leukocytes infiltration were evaluated (Histology) at 90 days.
Results: At 7 days, early Gevo limited the early LV expansion and reduction of FS induced by I/R. At 90 days both of early and delayed Gevo as well as perindopril limited in a similar manner, the LV late dilatation, the reduction of FS and LV systolic and diastolic dysfunction induced by I/R. At 90 days, GK coronary endothelium-dependent relaxation to acetylcholine was impaired by I/R (59 ± 13% vs.17 ± 4%, $P < 0.05$). Early, delayed Gevo and perindopril restored the (86 ± 4%, 92 ± 2% and 98 ± 1% respectively; $P < 0.05$ vs. GK + I/R) coronary relaxation to acetylcholine. Early, delayed Gevo and perindopril significantly reduced collagen density and leukocytes infiltration at 90 days.
Conclusions: In a clinically relevant model of acute myocardial infarction, the IL-1β antibody gevokizumab started early or late after myocardial reperfusion exerts immediate and late cardiovascular protection.
Keywords: IL1-beta antibody, ischemia/reperfusion, LV remodeling, coronary relaxation.

CO-020

TREK-1 potassium channel as a potential target to alleviate chronic neuropathy induced by Oxaliplatin-chemotherapy

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Oxaliplatin is platinum derivate that is used in the treatment of colorectal carcinoma (1). Administration of this drug produces cold-induced dysesthesia and paresthesia induced or exacerbated by cold at both extracephalic and cephalic territories immediately following the first infusion in 90% of patients, and about 20% of patients develop chronic dose-limiting neuropathy that compromises quality of life and survival (2). We have previously shown, using molecular, electrophysiological and behavioral experiments, that the potassium ionic channel TREK1 may represent a molecular target to alleviate acute hypersensitivity induce by a single dose of oxaliplatin (3). In the present study we first aimed to reproduce, in mice, patients' symptoms of oxaliplatin-induced chronic neuropathy. We also investigated TREK1 channel involvement in the pathophysiology of chronic oxaliplatin-induced neuropathy, and we use the non-specific TREK1 activator Riluzole to validate this channel as an interesting pharmacological target for the treatment of pain induced by chronic oxaliplatin administration.
Oxaliplatin (6 mg/kg, i.p) was administrated twice a week for 4 weeks in TREK-1 know-out mice and their wild-type littermates. Riluzole (60 μg/mL) treatment was administrated in the drinking water(4). Cold hypersensitivities were assessed by tail immersion at 10 °C and orofacial acetone test. Mechanical hypersensitivities were assessed using Von Frey test applied on the fore paws and orofacial brush test. Others symptoms clinically described as apraxia and sensibility disorders were assessed respectively with adhesive removal test (5) and beam walk (6).
The results clearly show that repeated oxaliplatin treatment mimics symptoms observed clinically at the extracephalic level and, first time in animals, at the cephalic level. These results, added to the sensorimotor deficits observed using the beam walk and adhesive removal tests, makes this model of clinical relevance. Riluzole partially alleviates cold and mechanical sensitivities, sensorimotor deficits in wild type animals but not in TREK-1 knock-out animals. Altogether, we developed an animal model of chronic oxaliplatin-induced pain neuropathy and demonstrated that the potassium channel TREK-1 represents a potential target to alleviate pain in this model.
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Keywords: oxaliplatin, chronic neuropathy, TREK-1, Riluzole.

CO-021

Hemolytic anemia, a novel severe adverse event related to natalizumab

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Introduction: Natalizumab is one of the most effective therapy for refractory relapsing remitting multiple sclerosis. A report of a very convincing case of natalizumab-induced autoimmune hemolytic anemia (AIHA) to our center prompted us to analyse the French Pharmacovigilance database (FPD).
Patients and methods: The FPD was explored up to November 2013 by using the MedDRA system organ classification '*Blood and lymphatic system disorders*' and natalizumab as a suspected drug. Cases were retained if they fulfilled 3 of the 4 following criteria: hemoglobin <120 g/L, decreased serum haptoglobin level, hyperbilirubinemia or reticulocytosis, or with strong clinical evidence of AIHA.
Results: Five cases were retrieved for analysis (2 men and 3 women with a mean age of 40.6 ± 14.7 years). The mean natalizumab duration to onset was 29.2 ± 18.2 monthly dose (10–52). When available, laboratory findings included: hemoglobin level <120 g/L in 4/5 patients (mean 79 g/L), serum haptoglobin level <0.08 g/L in 4/4, hyperbilirubinemia in 2/2 and reticulocytosis in 1/2. Only 1 patient presented with typical clinical symptoms of anemia (dizziness, weakness), whereas the others remained asymptomatic. Direct antiglobulin test (DAT) performed in 3 patients was positive in 2 (IgG and C3d positive in one; IgG negative and C3d positive in one). A drug-dependent anti-red cell antibody search was negative in the single tested patient. Natalizumab was discontinued in all but one patient and corticosteroid were initiated in 2. Full recovery was confirmed in 3 patients (outcome unknown in 2) after a minimum of 1.5 month of follow-up and negativation of DAT was observed in the 2 positive patients. In one patient, natalizumab reintroduction resulted in recurrence of hemolysis again associated with positive DAT. Natalizumab was definitively stopped and hemolysis spontaneously recovered in 7 months.
Discussion: Until now, AIHA is not mentioned as a possible adverse event related to natalizumab. As no more than 10 000 patients have been treated with natalizumab since its marketing in France, the incidence rate of natalizumab-induced AIHA could be estimated to 0.5/1000 users, which is a 50-fold increased risk compared to the risk of spontaneous AIHA.
Keywords: autoimmune hemolytic anemia, natalizumab, multiple sclerosis.

CO-022

Cetuximab acneiform eruption, a marker of efficacy for metastatic colorectal cancer and head-neck cancer?

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Cetuximab is a monoclonal antibody against Epidermal Growth Factor Receptor, indicated for metastatic Colo-Rectal Cancer (mCRC) and for head-neck cancer. Studies suggest that development of acneiform eruption adverse drug reaction (ADR) are associated to a better efficacy of the drug for mCRC patients, but only few data are available for head-neck cancer patients. Our work aimed to assess, in real use conditions, the association of cetuximab most frequent ADRs effect, acneiform eruption with the overall survival for mCRC and head-neck cancer.
We conducted a retrospective observational study at Claudius Regaud Institute, Toulouse. The overall survival was defined as the number of days between the first administration of cetuximab and the date of death (all causes combined). Multivariate survival analysis was performed by the Cox model.
Between February 2004 to June 2010, 97 patients with a mCRC and 70 patients with a head-neck cancer have been included. Median age was 60 years [54–69] for mCRC and 58 years [53–64] for head-neck cancer (NS). The sex ratio was respectively 1.1 and 4.0 ($P < 0.001$). The median number of cetuximab cycles was 6 [4–11] for mCRC and 4 [3–6] for head-neck cancer ($P < 0.02$). Acneiform eruptions were observed in 70 (72.2%) patients with mCRC vs. 40 (57.1%; $P < 0.02$), with a median time to occurrence of 15 days [14–23] for mCRC vs. 22 days [14–28] for head-neck cancer (NS). The median overall survival was 249 days [109–461] for mCRC and 176 days [117–346] for head-neck cancer (NS). In univariate analysis, acneiform eruptions were significantly associated with a relative reduction risk of death of RR = 0.34 [0.21–0.55], $P < 0.001$ for mCRC and RR = 0.28 [0.16–0.48], $P < 0.001$ for head-neck cancer. In multivariate analysis, eruptions were always associated with a better overall survival for

the two types of cancer (RR = 0.34 [0.17–0.37], $P < 0.002$ and RR = 0.38 [0.21–0.70], $P < 0.002$, respectively for mCRC and for head-neck cancer).
Despite a significant lower incidence of acneiform eruptions for head-neck cancer patients, we found for the first time that acneiform eruptions are associated to a better overall survival for head-neck cancer and that the risk reduction is comparable to mCRC.
Keywords: cetuximab, acneiform eruption, overall survival, metastatic colo-rectal cancer, head-neck cancer.

CO-023

Antineoplastic and immunomodulating agents use during pregnancy: a retrospective study in TERAPPEL database

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Objective: Few clinical data are available regarding the effects of antineoplastic and immunomodulating agents in pregnant woman. The objective of the present study was to describe the outcome of pregnancies exposed to 'antineoplastic and immunomodulating agents' using TERAPPEL database.
Material and methods: We performed a retrospective descriptive study using TERAPPEL, a French database which records requests from health professionals and patients to Regional Centres of PharmacoVigilance about women exposed to drugs during pregnancy and/or breastfeeding.TERAPPEL registers the outcome of pregnancy. We analyzed all cases of women exposed during pregnancy to 'antineoplastic and immunomodulating agents' (L01 to L03 ATC class) from 1984 to 2009.
Results: Fifty eight women exposed to 'antineoplastic and immunomodulating agents' during pregnancy were identified of whom 56.9% ($n = 33$) were exposed during the first trimester. The most common cancers were breast cancer ($n = 37$, 63.8%) and leukemia ($n = 7$, 12.1%). The most frequent used drug classes were alkylating agents ($n = 28$, 48.3%) among 'antineoplastic agents' (L01) and hormone antagonists and related agents ($n = 17$, 29.3%) among 'endocrine therapy' (L02). Immunostimulants (L03) were used in only 6.9% of cases ($n = 4$). The most cited drugs were cyclophosphamide ($n = 21$, 36.2%), epirubicin ($n = 17$, 29.3%), tamoxifen ($n = 17$, 29.3%), fluorouracil ($n = 17$, 27.6%) and doxorubicin ($n = 8$, 13.8%). The outcome of these pregnancies was as follows: 43 live-births (74.1%), 8 medical terminations (13.8%), 3 voluntary terminations (5.1%), 2 miscarriages (3.5%) and 2 intrauterine deaths (3.5%). The 4 (6.8%) newborns and fetus (2 medical terminations) with congenital malformations were exposed during organogenesis. Neonatal complications were evidenced in 13 neonates (29.5%), of whom 11 were exposed during the second and third trimesters. Cardiorespiratory diseases were the most common (9 cases) complications. One case of urinary tract infection, 1 hypothermia-hypoglycemia and tremors, 1 cervical tumefaction and 1 hypotonia were also observed.
Discussion: In our study, chemotherapy administered after the first trimester seems to imply little risk to the fetus. Data about 'antineoplastic and immunomodulating agents' during pregnancy should be notified and registered.
Keywords: antineoplastic and immunomodulating agents, pregnancy, TERAPPEL, congenital malformations, neonatal complications.

CO-024

Infections during the first year of life after in utero exposure to drugs acting on immunity: follow-up of children from the EFEMERIS database

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Introduction: Some studies suggest that antenatal exposure to immunosuppressive drugs is associated to alteration of immunity biological parameters. The objective was to evaluate the association between *in utero* exposure to drugs that potentially exhibit immunosuppressive activity and occurrence of infections during the first year of life.
Methods: We conducted a cohort study on prescription data of pregnant women and their children registered in the EFEMERIS cohort (Haute-Garonne; France), between the 1st of July 2010 and the 30th of June 2011. We classified children exposure in four categories: unexposed, one reimbursement of an immunosuppressive drug during the whole pregnancy, two reimbursements, three or more. The number of infectious episodes in the children's first year of life was estimated through the number of different dates of anti-infectious drug deliveries. The risk of developing infectious episodes was estimated by a Quasi-Poisson regression. Sex, season of birth, mother's age and number of drugs received by the mother during pregnancy were introduced in the model as confounding factors.
Results: Study population included 9614 children. Nasal glucocorticoids were the most reimbursed immunosuppressive drugs (3119 reimbursements, 52%) followed by systemic glucocorticoïds (1564 reimbursements, 26%) and inhaled glucocorticoïds (1158 reimbursements, 19%). The mean number of infectious episodes during the first year of life increased with the number of potentially immunosuppressive drugs delivered during pregnancy: 2.39 for unexposed children, 2.92 after one *in utero* exposure, 3.33 after two and 3.90 after three or more. After adjustment on confounding factors, *in utero* exposure was significantly associated with the number of anti-infectious drugs delivered during the first year of life (adjusted $RR_{1\ reimbursement\ vs.\ 0} = 1.12$; IC 95% [1.07–1.18]/$RR_{2\ reimbursements\ vs.\ 0} = 1.20$; IC 95% [1.12–1.29]/$RR_{3\ or\ more\ reimbursements\ vs.\ 0} = 1.35$; IC 95% [1.24–1.46]).
Discussion: Intrauterine exposure to potentially immunosuppressive drugs is associated with more infectious episodes during the first year of life. This pharmaco-epidemiological study seems to be in accordance with biological data showing an effect of immunosuppressants on immunity parameters.
Keywords: cohort study, pregnancy, children, immunosuppressive drugs, infections.

CO-025

Outcomes of mothers and children after cancer treatment before or during pregnancy: 3 years of experience in the French Regional Pharmacovigilance Center of Languedoc-Roussillon

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Objective: Treatment of cancer is improving life expectancy and pregnancies can start various times after chemotherapy. Cancer could also be diagnosed during pregnancy, although this is rare and then the question about treatment is raised, with regards to fetal safety and optimal efficacity for the mother.
Methods: We report 3 years of experience (2010–2012) in the French Regional Pharmacovigilance Center of Languedoc-Roussillon sollicited for counselling for 19 pregnancies in 18 patients, with special focus on outcomes of both mothers and children.
Results: 16 cases were collected prospectively and 3 retrospectively. Chemotherapy was involved in 16 cases, radiotherapy in 1 and tyrosine kinase inhibitor in 2. Treatments were administered during pregnancy in 12 cases: in 11 cases, between 18 and 33 SA, and in one case radioactive iodine was delivered at 5 SA (unknown pregnancy).
Outcomes were known in all cases: 2 elective abortions, 4 therapeutic abortions (1 because of fetal risk: radioactive iodine for thyroide cancer with estimated uterus dose (i.e. >200 mGy) during 1st month pregnancy; 1 for maternal risk; 2 because of the need for an aggressive treatment, without specific pregnancy data and poor maternal prognosis), 14 live births (one set of twins), without any congenital anomalies (one colonic duplication but detected before chemotherapy). One baby presented transient thrombocytopenia and increase transaminase level after birth (platelets: 50 000/mm^3 on Day 3 and 5000/mm^3 on Day 7, without any haemorrhage and recovering on Day 10; Amino Alanine Transferase 88 UI/mL on Day 3), after 6 cycles of FEC therapy (5-fluorouracil, epirubicin and cyclophosphamide), last one 5 weeks before birth. Monitoring of mothers and children is on going.
Conclusion: Until recently, pregnant women with malignant disease have mostly been advised to stop their pregnancies. However, treatments of cancer could in fact be considered for pregnant women after first trimester, with individual counselling, close monitoring and extended follow-up.
Keywords: pregnancy, cancer, chemotherapy, exposed mothers and children, follow-up.

CO-026

Outpatient use of oral anticancer drugs in the permanent random sample of the French healthcare insurance database (2006–2011)

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Objective: Estimate use of oral anticancer drug in outpatients from data of the permanent random sample of the French healthcare insurance database from 2006 to 2011 and describe the characteristics of treated patients.
Methods: Repeated cross-sectional study from 2006 to 2011 in the 1/97th permanent random sample of the French national healthcare insurance database (*Echantillon Généraliste de Bénéficiaires,* EGB). Drugs of interest were all oral anticancer drugs identified in the database using the Anatomical Therapeutic Chemical classification. For each year from 2006 to 2011, the prevalence (at least one dispensation for a given subject) and the incidence (at least one dispensation but none during the previous year) of oral anticancer drug dispensations in outpatients were estimated.
Results: The annual prevalence of oral anticancer drug use in the EGB database increased from 3727 patients in 2006 to 4094 in 2011. During the same period, there was little variation of annual incidence use: 1415 patients in 2006 and 1394 in 2011. Incidence of oral cytotoxic chemotherapy use was 232 patients in 2006 and 274 in 2011; of oral targeted therapy: 22 and 146; of oral hormonal anticancer drugs: 1218 and 1034. For incident users, there was little variation in age (median [interquartile range]: 60 years [40; 74] in 2006, 62 years [46; 75] in 2011), in sex ratio (68% female in 2006, 69% in 2011), or in administrative registration for cancer (*Affections de Longue Durée*, ALD: 46% in 2006, 48% in 2011).
Discussion: The annual prevalence of oral anticancer drug use in outpatients increased from 2006 to 2011; annual incident use was relatively constant. Less than half of incident users had administrative registration for cancer, which suggests that precaution should be taken when using this for identification of cancer patients. For pharmacoepidemiological studies of these drugs the high frequency of oral hormonal anticancer drug use suggests that the EGB database could be considered; the infrequent use of oral targeted therapies and oral cytotoxic chemotherapy suggests that the full health insurance database (SNIIR-AM) should be employed.
Keywords: oral anticancer drugs, French healthcare insurance database.

CO-027

Anaphylaxis to thymoglobulin: an undesirable rabbit

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Introduction: Thymoglobulin is an anti-thymocyte globulin prepared from rabbits immunized with human T lymphocytes, and used in the prevention and treatment of acute transplant rejection. Hypersensitivity reactions to anti-thymocyte globulin mostly consist of serum sickness, whereas immediate anaphylaxis has been only reported as isolated case reports. We describe a case of anaphylactic shock to anti-thymocyte globulin in a patient previously exposed to a domestic rabbit.

Case report: A 25-year-old man with end-stage renal disease due to Alport syndrome was referred for renal transplantation. After uncomplicated anesthetic induction with remifentanil, propofol and cisatracurium, the drug regimen consisted of anti-thymocyte globulin, ceftriaxone, gentamicin, omeprazole, vitamin C, and mycophenolate mofetil. Within a few minutes, the patient experienced grade-3 anaphylaxis with cardiovascular collapse and generalized maculopapular rash requiring epinephrine and intravenous fluids. As he gradually recovered normal vital signs, the surgery was finalized. Ten and 2 h after the acute reaction, serum histamine levels were higher than 100 nM, and serum tryptase levels were measured at 127 μg/L and 71 μg/L, respectively. Skin prick tests performed 10 weeks later with all the drugs received during surgery were only positive for anti-thymocyte globulin. On further questioning, the patient indicated that he suffered from allergic rhinitis since her family had bought a rabbit living at home. Further skin prick tests performed with commercial rabbit fur were strongly positive as were specific IgE to rabbit serum proteins.
Discussion: To our knowledge, anaphylaxis to anti-thymocyte globulin resulting from sensitization to rabbit serum proteins after direct exposure to the animal has not been reported previously. The major rabbit allergen involved in anaphylactic reactions has been identified as a glycoprotein (*Oryctolagus cuniculus*). Based on this case, we strongly recommend a careful patient history including any signs of hypersensitivity to rabbit before using rabbit-derived anti-thymocyte globulin.
Keywords: thymoglobulin, anaphylaxis, rabbit, sensitization.

GS4 ENDOCRINO

CO-028
Immunolocalization of matrix metalloproteinases in the testis and vas deferens of the Libyan jird (*Meriones libycus*)
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This study was undertaken in order to investigate the involvement of matrix metalloproteinases (MMPs) in the reproductive physiology of a nocturnal Saharan rodent, the Libyan jird *(Meriones libycus*). An immunohistochemical study of matrilysin-1 (MMP-7) and gelatinase B (MMP-9) using the indirect method with streptavidin biotin peroxidase was performed on the testis and vas deferens of *Meriones libycus* collected from its natural habitat (Béni-Abbès, Algerian Sahara) during the breeding season (spring and early summer). In seminiferous tubules, MMP-7 was highly expressed in the three cell types that reside in the basal compartment, spermatogonia, spermatocytes I at preleptotene stage and Sertoli cells. The other germ cells located in the adluminal compartment show a slight immunoexpression; MMP-7 immunolabeling was observed on sperm head and not on the flagellum. In the testicular interstitial space, Leydig cells show a significant immunostaining. MMP-9 immunolabeling was slightly lowered and extremely repressed at the base of the basal compartment and is found only in Sertoli cells and spermatogonia. In the other germ cells and Leydig cells the immunoreaction was absent; only spermatozoa were positively immunostained. In the vas deferens, the immunolabeling pattern of both MMPs is similar, muscular wall formed of smooth muscle cells (SMCs) and the epithelial cells were strongly immunolabeled. No immuno-response was observed in the lamina propria and the extracellular matrix infiltrated between SMCs. In the epithelial fraction, immunostaining of MMP-7 is diffused, occupies the entire cytoplasm and is also observed on the apical microvilli and apocrine secretory vesicles. MMP-9 immunostaining was mainly in basal and apical positions without any immunostaining on the apical microvilli and apocrine secretory vesicles. Both MMPs could have an important function in the physiology of the reproduction of *Meriones libycus* such as detachment of spermatogonia from basement membrane, blood testis barrier restructuring to allow germ cells transition from basal to adluminal compartment during the spermatogenesis (Chen et al., 2012), spermiation and fertilization (Ferrer et al., 2012) and in the vas deferens physiology (Cardoso et al., 2010).
Keywords: matrix metalloproteinases, testis, vas deferens, MMP-7, MMP-9, spermatogenesis, blood testis barrier, Sertoli cells, Leydig Cells, germ cells.

GS5 NUTRITION

CO-029
Gustatory evoked cortical activity in humans in response to saccharin stimuli
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Introduction/Objectives: Gustatory Evoked Potentials (GEP) can be detected in response to an intermittent stimulation of the gustatory receptors by a primary flavour. Contrary the other evoked cortical potentials (visual, auditory or sensory), GEP have not been extensively studied due to their recording heterogeneity. Our aim was to establish a reliable recording of GEP in Humans.
Methods: Voluntary and healthy subjects were included in this experiment. A saccharin solution with several concentrations (5, 10 or 20 g per 100 mL) was used as the gustatory stimulus. Each session was performed on a different day, with an interval of at least 2 h after eating or drinking. Control session was performed using water or no stimulus. The saccharin solution was presented to the median part of the tongue by intermittent stimulation. The intermittent stimuli were monitored by a specific equipment built for this purpose. Each 1s stimulus was presented 20 times with an inter-stimulus interval of 1 min water. Each session was repeated twice. GEP were recorded from 9 cortical sites with EEG sensors: Cz, Fz, Pz, C3, C4, F3, F4, Fp1 and Fp2 of the 10/20 system (referenced against linked earlobes). GEP were obtained after average of all the responses.
Results: Fifteen healthy subjects participated in this experiment: 10 women and 5 men, from 25 to 63 years old (mean age 34). GEP consisted in one negative wave, without differences between men and women: their latency varied from 140 to 181 ms and their amplitude fluctuated from 7 to 24 μV. There is a good inter- and intra-individual reproducibility. None GEP was obtained during the control sessions. The others experiments (stimuli by different saccharin concentrations) are in progress.
Discussion/Conclusion: These data demonstrate that record GEP in Humans in response to saccharin stimuli is reliable. These data are in good agreement with the previous works. Studies are currently conducted to assess GE cortical activity in response to other primary flavor and to determine the effect of satiety on these parameters.
Keywords: gustatory evoked potentials, saccharin solution.

CO-030
CD36 AA single nucleotide polymorphism (SNP) is associated with decreased lipid taste perception in Tunisian obese women: association with pro-inflammatory TNF-a GA and IL-6 GC genotypes
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Objective: Common single nucleotide polymorphisms (SNPs) play an important role in many pathological states, including obesity. The aim of the study was to assess whether CD36 SNP along with with TNF- α and IL-6 polymorphisms are implicated in obesity.
Methods: A total of 196 obese Tunisian women were recruited for this study. All the subjects underwent an anthropometric measuring (weight, height, BMI) and a morning blood sample (fasting state) was drawn for further analysis. The blood plasma was used for determination of fasting glucose and glycosylated haemoglobin (HbA1c) levels. Serum was used to assess additional physiological parameters (triglycerides, cholesterol, insulin, urea, creatinine, IL6, TNF-α).The alternative-food choice (AFC) method was apply to determine free fatty acid (FFA) oral sensitivity. Oleic acid in different concentration was used for this experiment. DNA samples obtained from the blood were used for determination of genotypes of 3 SNPs (rs1800629- TNF-α, rs1800795-IL-6, rs1761667-CD36).
Results: We observed significant association between higher creatinine concentration and GG genotype of rs1800629 ($P < 0.05$). GG genotype of rs1800795 was correlated with high concentration of IL-6 in serum ($P < 0.02$). AA genotype of rs1761667 was associated with high concentration of cholesterol ($P < 0.01$) and LDL ($P < 0.01$) in serum. Statistical significance was also noticed between GG genotype in rs1761667 and high HbA1c level in the blood serum. In additional this genotype was strongly associated with higher oral sensitivity for oleic acid ($P < 0.001$).
Conclusion: Our study confirms, for the first time, high thresholds of fat detection in obese subjects, expressing CD36 AA genotype without having any significant correlation with the SNPs of IL-6 and TNF-α genes.
Keywords: CD36, fatty acid, IL-6, obesity, SNP, taste, TNF-alpha.

CO-031
Nutrition transition in Constantine: nutritional profile
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Nutritional objectives of this study imposed the use of tool to qualify and quantify nutrient intakes. View the constraints imposed by semainiers due to the increase in collections, we opted for collections of 3 days can be tracked while limiting constraints for the 500 participants. It's Friday, Sunday and Tuesday, this collection is repeated five times to identify the specific food recruited.
The results of this survey show that 90% of participants eat unbalanced and undiversified. The diet is rich in animal products, fats, sugars, causing deficiencies in the intake of dietary fiber, vitamins and especially many trace elements. They eat a lot of bread, potatoes, pasta and sugary drinks. 80% of young people eat too much fat products such shawarma, mahjouba, kabab, pizza accompanied by sugary soft drinks. All participants drink very little water <500 mL/day.
Fruits and vegetables are 'under – consumption', with an average of 260 g per adult per day. It is necessary to swallow at least 400–800 g/day. However garlic and onions are rich in sulfur compounds that may have anti-cancer properties and interesting protective effects in the cardiovascular level is consumed regularly for lunch and dinner it is the same for spices that are likely to have beneficial effects on the body.
10% of recruited eat meat once every 3 days. 50% of participants opted more often for poultry and only 2% consume fish. 60% of participants consume more than 6 g of salt per day.
Most participants have very poor eating habits. Premature aging of these subjects and identified common diseases (diabetes, hypertension, hypercholesterolemia) are major arguments for recommending abundant plant products consumption and justifies the need to encourage them to provide sufficient antioxidant protection to their organism. This recommendation should focus on a very diversified products of good quality nutritional intake.
Keywords: nutrition, diet, fats, eating habits, constantine.

CO-032
The effect of acute maximal exercise on plasma concentration of ghrelin during Ramadan Fasting

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Introduction: The peptide hormone ghrelin is an important stomach hormone that influences several metabolic activities (Ejskjaer et al. 2010; Nogueiras et al. 2008). We hypothesized that Ramadan fasting could affect the plasma concentrations of ghrelin during a short-term maximal exercise.
Methods: Eleven male soccer players (age, 20.6 ± 1.3 yrs; height, 176.6 ± 2.5 cm; body-mass, 71.1 ± 8.6 kg; mean ± SD) performed a Wingate test on three different occasions: (i) 1 week before Ramadan, (ii) the first week of Ramadan, and (iii) the fourth week of Ramadan. Blood samples were taken before, immediately and 60 min after the exercise.
Results: Our results showed significant increase of plasma concentrations of ghrelin during Ramadan ($P > 0.05$). However, no significant effect of Ramadan on Ppic (PP) and Pmean (PM) during the three testing periods was observed.
Conclusion: In conclusion, our study shows that ghrelin's is an important regulator controlling glycemia under fasting conditions.
Keywords: exercise, Ghrelin, Ramadan fasting.

CO-033
Antiproliferative effect of carob polyphenolic extract (pulp and leaves) in HCT116 and HT29 colon cancer cell lines

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Objective: The aim of this study was to check the antiproliferative effect of phenolic compounds derived from different parts of carob, pulp and leaves, on HCT 116 and HT29 colon cancer cell lines.
Material and methods: Vegetal materials were collected from Tlemcen region (Algeria). The samples were grounded then polyphenols were extracted according to the method followed by Liyana-Pathirana and Shahidi (2006). The cells were seeded in Petri dishes (2 mL) and incubated with plants polyphenolic extract and Gallic acid as a positive control during 24 h and 48 h at 37 °C and 5% of CO_2.Cell viability was determined by trypan blue exclusion test and by MTT assays. Antiproliferative mechanism of leaves was evaluated by flow cytometry by using fluorescent markers 7-AAD (necrosis indicator) and annexin V (apoptotic indicator).
Results: MTT and Trypan Blue assays showed that the phenolic extract of leaves extract significantly decreased the proliferation of both HCT 116 and HT29 in a dose and time dependent manner. Nonetheless this inhibition was more significant on HT29 cells, as phenolic extract of leaves induced 80% inhibition of proliferation at the concentration 30 µg/mL. Furthermore, phenolic extract of pulp exerted a weak inhibitory effect when compared to leaves of carob. Phenolic extract of leaves induced both necrosis and apoptosis as this compound increase both 7-AAD permeability and externalization of phospholipid phosphatidylserine (FITC Annexin V staining).
Discussion: The selective antiproliferative effect of leaves extract may be related to its content of phenolic compounds.This suggest that leaves from carob tree could be a source of phenolic compounds with potential anticancer activity.
Keywords: antiproliferative effect, carob, phenolic compounds, HCT116 and HT29.

CO-034
Relationship between eating behaviour disorders, obesity and type 2 diabetes: effects of hormonal corticotropic axis and visceral adiposity

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Introduction: The metabolic syndrome is involved in various human pathologies including visceral adiposity and insulin resistance in type 2 diabetes. In obesity, increased body weight is frequently associated with excessive caloric food intake and sedentary. The Behaviour Eating Disorders (BEDH) is involved in overconsumption alimentary. In Algeria, the eating behaviour has been increasingly deviated from the Mediterranean diet to fast-food model.
Objective: The present study examines the interactions between the Behaviour Disorders related to Eating Habits (BDEH), corticotropic hormone axis and metabolic syndrome.
Patients and methods: Algerian population cohort was composed of 410 subjects (130 obese, 170 type 2 diabetics and 110 healthy subjects). The BDEH was evaluated by Three Factor Eating Questionnaire test (TFEQ). Anthropometric parameters (waist circumference and Body Mass Index), metabolic parameters (glucose, triglycerides, HDL-c, LDL-c and cholesterol) and the levels of hormones (insulin, cortisol, ACTH and GH) were determinated by biometrics spectrophotometry and radioimmunoassay respectively.
Results: Compared to healthy subjects, obese ones showed hyperphagic type of BDEH (disinhibition and hunger disorders). Conversely, the diabetic patients showed both hypophagic and hyperphagic BDEH. Multivariate analyses showed the high correlation between BDEH and metabolic syndrome, particularly between a critical insulinoresistant state and the BDEH. This insulinoresistance generates metabolic disorders: dyslipidemia, hyperglycemia and hypertrophy of adipose tissue. In diabetic and obese subjects, cortisol and ACTH secretions were significantly altered, leading metabolic disorders.
Conclusion: Our study confirms the role of the BDEH in obesity and diabetes. Besides, in response to nutritional stress, the BDEH seems to trigger the hyperactivity of pancreas, adrenal and pituitary glands.
Keywords: metabolic syndrome, insulin resistance, BDEH, type 2 diabetes, obesity.

CO-035
Immunomodulating effect of Pearl Millet's (*Pennisetum glaucum*) crude fat: in vitro by means of splenocytes proliferation assay and measurement of $[Ca^{2+}]i$

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Objective: This work aimed to assess the proliferative response of splenocytes to *Pennisetum glaucum* crude fat (PGCF): *in vitro* by means of lymphocyte transformation test (LTT) and measurement of free intracellular Ca^{2+} concentrations ($[Ca^{2+}]i$).
Materials and methods: Grains of millet cultivar (*Pennisetum glaucum*) were obtained from the South of Algeria. Crude fat were extracted according to the method of Hara and Radin (1978); Isopropanol and hexane were the solvents used for the conventional solvent extraction. Splenocytes were harvested aseptically from *Wistar* rat and suspended in RPMI 1640. The effect of PGCF on lymphocyte proliferation was performed by WST-8 Cell Proliferation Kit (Cayman Chemical). The effects of PGCF on intracellular calcium concentration ($[Ca^{2+}]i$) was performed after loading splenocytes with the specific Ca^{2+} florescent probe, Fura-2/AM, and intracellular Ca^{2+} response to different concentrations of PGCF (from 1 to 40 µg/mL) was performed on PTI spectrofluorometer at 340 and 380 nm (excitation filters) and 510 nm (emission filters).
Results: We observed that PGCF evoked a dose dependent $[Ca^{2+}]i$ increase in spleen cells. We used PMA plus Ionomycin to induce T cell proliferation. We observed that PGCF significantly curtailed PMA + Ionomycin-induced splenocytes proliferation, However PGCF alone failed to modulate proliferation of non-stimulated splenocytes.
Discussion: The effect of PGCF seems to be similar to the finding of Denys et al. (2005), who have shown that n-3 polyunsaturated fatty acids (EPA and DHA) increased $[Ca^{2+}]i$ and inhibited both PMA-induced T cell proliferation and nuclear translocation of NF-κB, however these fatty acid exerted no significant effect on non-stimulated cells.
Keywords: *Pennisetum glaucum*, crude fat, splenocytes, LTT, $[Ca^{2+}]i$.

CO-036
Knowledge, attitudes and practices of mothers about feeding preschoolers

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Introduction: We are witnessing the last few decades a real upheaval in feeding behavior explains, therefore, the epidemiological transition knows our society. Children in this situation do not seem safe because the family and social environment has an influence on their behavioral attitudes towards food. It is for this purpose that we want to evaluate the knowledge, attitudes and practices of mothers towards children nutrition and develop an educational guide.
Methodology: This is a descriptive cross-sectional study, which included 60 mothers of children aged 3–5 years, enrolled in different kindergartens in the region of Tunis from 16 January to 16 February 2012. Data collection was conducted using a questionnaire in French, anonymous, directed, interview type, divided into four items, pre-tested, relating to the identification, general information about the mother and her child, knowledge, attitudes and practices of mothers about feeding their children. We then measured the weight and height of children, to calculate BMI and classify them according to their body size.
Results: BMI children showed a higher than normal body size (26% of cases) and lower than normal (17% of cases). A healthy breakfast is consumed only in 3.3% of children, while 98.3% of them take a morning snack based on bakery and confectionery trade. The snack in the afternoon, although including dairy products is also based on bakery products and trade at very high energy density. The concept of a balanced diet is not well respected in most mothers' behavior. Moreover, the best mode of cooking enjoyed by children is represented by frying. In our study, a normal food pace (3 meals and a snack in the afternoon) is found only in 1.7% of children.
Discussion: A dietary imbalance associated with a lack of rhythmicity in taking meals were observed in these preschoolers. This justifies the interest of nutritional and behavioral education of children. It is in this context that we proposed an educational guide for this purpose.
Keywords: dietary imbalance, child in preschool, Tunisia.

GS6 SOMMEIL

CO-037
Role of non-muscular myosin light chain kinase (nmmlck) in the inflammation associated with intermittent hypoxia

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Introduction: Obstructive Sleep Apnea Syndrome (OSAS) is characterized by repetitive obstructions of the upper airway during sleep, inducing intermittent diminution in oxygen saturation, or intermittent hypoxia (IH). IH alters endothelial function favoring inflammation and could accelerate atherosclerosis-induced cardiovascular diseases. A protein that may play a role in this process is the non-muscular myosin light chain kinase (nmMLCK). The deficiency of this kinase protects mice from death in septic choc models and prevents the atherosclerosis in mice fed with a high fat dietary.
Objective: The aim of this study was to analyze the implication of nmMLCK in the vascular effects and the inflammation induced by IH.
Materials and methods: Human aortic endothelial cells (HAoECs) and endothelial cells isolated from wild type or deficient in nmMLCK mice were exposed to 6 h of IH, which include 30 min of hypoxia (O_2 5%) followed by 30 min of normoxia (O_2 21%), in the absence or the presence of ML-7 (5 µM), a nmMLCK inhibitor. After the stimulation, we evaluated the production of the superoxide

anion, nitric oxide (NO), the pro-inflammatory cytokine IL-6, and also the activation of the NF-kB pathway (p65).
Results: IH treatment increased superoxide, nitric oxide and IL-6 production in HAoECs. However, while the nmMLCK inhibitor ML-7 had no effect on the IH-induced superoxide anion increase, it decreased both NO and IL-6 production. Furthermore, p65-NF-kB pathway was activated by IH in a ML-7-insensitive manner in HAoECs. By using aortic endothelial cells from nmMLCK deficient mice, we showed that nmMLCK deletion abolished increase of superoxide anion induced by IH indicating that the presence and not the kinase activity of nmMLCK is crucial to regulate oxidative stress induced by IH.
Conclusion: IH induced oxidative and nitrative stresses as well as changes in inflammatory secretome of human endothelial cells. In this experimental model of IH, nitrative and pro-inflammatory cytokine secretion are sensitive to inhibition of the activity of the kinase and oxidative stress require its presence. These results suggest strength the notion that nmMLCK participate to the IH-induced inflammatory process in endothelial cells.
Keywords: intermittent hypoxia, nmMLCK, inflammation.

CO-038

Maintenance of wakefulness test: an electro-physiological measure to better phenotype ADHD adult patients

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Introduction: Patients with Attention Deficit Hyperactivity Disorder (ADHD) frequently report sleep disorders but no study has yet explored the level of objective sleepiness in adult ADHD patients. In this study, we used the maintenance of wakefulness test (MWT) to quantify the objective level of sleepiness of these patients and relate it to cognitive performance.
Methods: 40 adult ADHD patients and 19 healthy controls performed a nocturnal polysomnography (PSG), an MWT (4 × 40 min trials), and a 1-h simulated driving session. The primary outcome was mean sleep latency on the MWT. Patients and controls were divided into 4 groups defined by their MWT mean sleep latency scores. The groups were: sleepy ADHD (0–19 min), intermediate ADHD (20–33 min), alert ADHD (34–40 min) and control group (34–40 min). Driving performance was assessed with the Standard Deviation of Lateral Position of the vehicle.
Results: 14 of our ADHD patients (35%) were in the sleepy group, 20 (50%) were in the intermediate group, and only 6 (15%) were in the alert group. Only 7 ADHD patients presented nocturnal abnormalities on the PSG (AHI > 10/h and/or periodic legs movements > 15/h). Sleepy ADHD patients exhibited significantly deteriorated driving performance compared to the other 3 groups ($P < 0.01$).
Conclusion: This is the first study to show that a significant proportion of adult ADHD patients exhibit objective daytime sleepiness. Sleepiness, a symptom objectively measurable by MWT, may be a key element to better phenotype these ADHD patients.
Keywords: attention deficit hyperactivity disorder, maintenance of wakefulness test, sleepiness, sleep disorders, cognition, driving performance.

CO-039

Atypical sleep in ICU: Role of hypercapnia and sleep deprivation

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Patients in intensive care units experience severe sleep alterations. Nearly one third of them display a particular pattern of sleep that has been termed 'atypical sleep'. It has been associated with poor outcome in patients treated with non invasive ventilation.
Our definition of atypical sleep relies on the absence of sleep spindles and K-complex. We hypothesize that the disappearance of sleep spindles is a progressive process and that there is a continuum from normal sleep EEG to atypical sleep. We expected to find a correlation between spindle density and causal factors like hypercapnia and sleep deprivation. We determined the spindle density in PSG recordings of ICU patients displaying normal sleep EEG (ie without atypical sleep).
Aim: Assessing the impact of hypercapnia and sleep deprivation on the spindle density in patients without drugs and sepsis.
Material and methods: We reviewed the PSG recordings from 12 adults who were admitted to the medical ICU for acute respiratory failure and treated with non invasive ventilation during at least 2 days, and enrolled in a previously published study. PSG were performed on the 3rd night after admission. Non inclusion criteria included encephalopathy, sedative drugs administered within the last 48 hrs and known neurologic or psychiatric disease. Sleep was scored using conventional rules (AASM 2007) and spindles were visually counted independently by two experts. Blood gas was performed the morning after PSG. Sleep deprivation was estimated by quantifying EEG slow wave activity (power spectral of the delta band [0.5–4 Hz]) which increases with sleep deprivation.
Results: Spindles density ranges from normal value (1.8/min) to very low value (0.02/min) in our sample of 12 patients. Non parametric Spearman correlation analysis showed that spindle density was significantly correlated to PCO2 (rho = 0.5; $P = 0.02$). There was also a non significative trend suggesting a correlation between spindles density and SWA in these preliminary results. Spindles density was neither correlated with age nor arterial PO2.
Discussion and conclusion: Atypical sleep could be due to a combination of hypercapnia and sleep deprivation. Further studies are required to confirm these data on a larger group with a direct measure of sleep deprivation.
Keywords: ICU, atypical sleep, PSG (polysomnography), poor outcome, spindles, hypercapnia, sleep deprivation.

CO-040

Role of orexin in vulnerability to sleep deprivation in mice

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Sleep deprivation affects very differently normal subjects and homeostatic pressure or chronotype do not fully explain resilience or sensitivity to sleep loss. Alerting systems are good candidates to explain how sleep deprived subjects can stay awake during extended wakefulness. We decided to test the role of orexin system in maintenance of performances in normal sleep deprived mice.
Method: We performed an Ip injection of Alomorexant (ALX) (orexin receptor antagonist) to mices to measure its effect on Reaction Time (RT) after sleep deprivation. Measure of RT was performed in mice in operant chambers, conditioned to press on a lever as soon as light is on to get food reward
Results:
1. We were able to discriminate between vulnerable and resistant mice to SD.
2. In vulnerable group, RT performance were not modified after ALX injection.
3. Resistant group show RT performance equivalent to vulnerable group after ALX injection.
Conclusion: Orexin release is involved in inter-subject variability to sleep loss. This finding is very important to open new research lines in development of alerting drugs but also possibly to select subjects that could be sensitive to specific alerting drugs. Histamine systems are good candidates to be tested in the future to confirm how different alerting systems play a role in maintenance of performance during sleep deprivation.
Keywords: sleep deprivation, orexin, reaction time.

CO-041

Cognitive characteristics of children with narcolepsy

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Objective: To conduct a descriptive analysis of cognitive characteristics in children and adolescents with narcolepsy.
Subjects and method: Clinical and electrophysiological characteristics of *de novo* patients from the Pediatric Lyon's Reference Center for narcolepsy were collected from 2008 to 2013. Due to the high frequency of school difficulties, intellectual ability (WISC-IV including full scale (Full IQ), verbal comprehension index (VCI), perceptual reasoning index (PRI), processing speed index (PSI), working memory index (WMI)) was usually proposed after the diagnosis. Some of these children were already treated at this time with stimulants (modafinil or methylphenidate).
Results: The cohort included 56 children (35 boys) with a median age of 12 years (range 5–17) (51.7%, <10 years). All children presented with EDS, 84% with cataplexy, 44.6% with hypnagogic hallucinations and 14.3% reported sleep paralysis, 64.3% were obese. 57% of the children had school difficulties, 29% repeated a year. 39 children were evaluated with WISC-IV. No differences were found between the tested and non tested children for clinical and polygraphic characteristics. Fourteen out of the 39 tested children (35.9%) were gifted, essentially with VCI > 130. The gifted children came from high social levels ($P < 0.001$), had more spontaneous arousals on PSG ($P = 0.01$) and were more often treated with stimulants (98% vs. 52% ($P = 0.025$)). VCI and Full IQ scales were correlated with social levels, spontaneous arousals, presence of treatment and school achievement. PRI was correlated with REM sleep% ($r = 0.50$, $P = 0.002$), REM sleep duration ($r = 0.46$, $P = 0.004$) and the presence of cataplexy (93 ± 10.4 for NwC and 107.7 ± 13.8 for NC, $P = 0.009$). A negative correlation was found between AHOI and PRI ($r = -0.37$, $P = 0.025$), WRI ($r = -0.42$, $P = 0.015$) and PSI ($r = -0.51$, $P = 0.002$). Low processing speed index was related to school difficulties ($P = 0.02$).
Conclusion: In these preliminary results, we found an interesting relation between the perceptual reasoning ability and REM sleep% as well as abnormal REM manifestations such as cataplexy. Further studies have to be made to confirm these results and to understand the underlying mechanisms. In the other hand, this study pointed out the negative influence of obstructive breathing on the WISC-IV results.
Keywords: narcolepsy, sleep, children, cognitive performances.

GS8 EXERCICE

CO-042
Muscle immobilization decreases single-fiber myosin heavy chain polymorphism

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Introduction: Immobilization of muscle leads to modification in its contractile phenotype. Myosin heavy chain (MHC) isoforms are the major determinant of the contractile phenotype of skeletal muscle. We therefore sought to evaluate the effects of immobilization on muscle MHC composition and polymorphism at single-fiber level.

Methods: The distal tendon of Wistar rat *Peroneus Longus* (PL) was cut and fixed to the adjacent bone at neutral muscle length. 4 weeks after the surgery, immobilized and controlateral PL were dissociated using enzymatic method (collagenase 0.3%) and the isolated fibers were sampled. MHC composition of single fibers was assessed using the sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) method (Caiozzo *et al.*, 2003; Rannou *et al.*, 2009 [1,2]). p38 phosphorylation was measured in 6- and 15-days immobilized PL and compared with controlateral muscle.

Results: MHC composition was as follows: I = 0%, IIa = 12.3%, IIx = 52.2%; IIb = 35.5%, in immobilized and I = 5.3%, IIa = 22.6%, IIx = 47.5%, IIb = 24.6% in controlateral PL. According to the Hill's statistical model of the force-velocity relationship (Hill, 1970 [3]), the MHC composition in immobilized muscle is consistent with a faster contractile phenotype compared with controlateral muscle. MHC analysis at the single-fiber level shows a polymorphism index of 31 and 39% in immobilized and controlateral muscles, respectively. An increase in p38 phosphorylation (1.6 fold) was observed following 6 and 15 days of immobilization (1.4 and 1.6 fold, respectively).

Conclusion: Despite the preservation of neural influences, single muscle immobilization at neutral length induces a shift of MHC composition toward a faster phenotype. The decrease in the proportion of hybrid fiber may result from the restriction in the length variations of immobilized muscle.

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Keywords: muscle immobilization, Myosin Heavy Chain, single-fiber polymorphism.

CO-043
Diagnostic algorithm for myoadenylate deaminase deficiency based on metabolic exercise testing parameters: a prospective study

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Introduction: The definitive diagnosis of metabolic myopathies requires an invasive muscle biopsy and subsequent highly specialized analysis. Our objective was to evaluate the accuracy of blood metabolites levels sampled during an incremental exercise test to diagnose the most common metabolic myopathy, that is myoadenylate deaminase (MAD) deficiency [1].

Methods: From December 2008 to September 2012, all the consecutive patients that both underwent a metabolic exercise testing and a muscle biopsy were prospectively enrolled. Subjects performed an incremental and maximal exercise testing on a cycle ergometer. Lactate, pyruvate, and ammonia concentration were determined from venous blood samples drawn at rest, during exercise (50% predicted maximal power output, peak exercise), and recovery (2, 5, 10, and 15 min). MAD activity was determined using *p*-nitro blue tetrazolium staining in cryostat sections from open muscle biopsy [2]. In order to generate a decision tree to identify absent and decreased MAD activity, we analysed the diagnostic accuracy of plasma metabolites using receiver operating characteristic (ROC) curves analysis [3].

Results: Among the 51 patients enrolled, McArdle and Tarui diseases were diagnosed in 2 and 1 subjects, respectively. Omiting glycolysis defect, MAD staining was absent in 5, decreased in 6, and normal in 37 subjects. Lactate/rest at the 10th minute of recovery provides the greater area under the ROC curves (AUC, 0.981 ± 0.044) to differentiate Absent from Present MAD activity. In this latest group, the pyruvate/ammonium ratio at the 5th minute of recovery from exercise displays the best AUC (0.871 ± 0.096) to discriminate between Decreased and Normal MAD activity. By combining the two biomarkers, the resulting decision tree achieves a diagnostic accuracy of 92.9%.

Conclusion: The present algorithm provides a non-invasive and accurate test to assess MAD deficiency, contributing to select patient for muscle biopsy and target appropriate histochemical analysis.

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Keywords: myoadenylate deaminase deficiency, metabolic exercise testing, diagnostic algorithm, ROC curves.

CO-044
Effect of whole body exercise duration on diaphragmatic central fatigue occurrence

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The present study was designed to test the effect of whole body exercise duration on the diaphragmatic central fatigue occurrence. Nine physically active male subjects performed three cycling exercise bouts requiring 55% of maximal oxygen uptake, and lasting 5, 15 or 40 min. Ventilatory parameters, transdiaphragmatic pressure (Pdi) and electromyographic activity of the sternocleidomastoïd (SCM) were monitored during exercise. The absence of peripheral diaphragmatic fatigue was checked with Pdi and mouth pressure responses to cervical magnetic stimulations, performed at rest and 15 min post exercise. Motor evoked potentials (MEP) were recorded from the diaphragm and the quadriceps in response to transcranial magnetic stimulation (TMS) at baseline and 10, 20 and 40 min following exercise. The MEP amplitude of the diaphragm and quadriceps remained unchanged after the exercises lasting 5 and 15 min, whereas it significantly decreased after 40 min of exercise. Our study confirms that diaphragmatic central fatigue can be present after a moderate exercise which lasts 40 min.

Keywords: excercise, central fatigue, diaphragm.

CO-045
Reliability of GPS measurements to assess walking limitation in patients with intermittent claudication

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Aim: The Global Positioning System (GPS) has been proposed as a valid measure to assess walking limitation in patients with peripheral artery disease (PAD). Nevertheless, the reliability of the related GPS-derived parameters that can be computed remains to be studied.

Material/Patients and method: Twenty PAD patients accepted to perform a test-retest stroll procedure. Patients were equipped with a GPS device (GlobalSat® DG100,Taiwan). Then, in a pre-defined public park, they were instructed to perform a 1 h outdoor stroll (T1), including stops duration, and only to stop for pain reasons in the lower limbs. A second stroll (T2) was performed 10 ± 5 days after T1 in the same park. The reliability, between T1 and T2, was assessed from the intra class coefficient of correlation (ICC) and the coefficient of variation (CV), both expressed with confidence intervals (CI) at 95% (CI 95%).

Results: A very high correlation was found between the 'highest measured distance between two stops during community walk' (HMD_{CW}) measured on T1 and T2 (ICC: 0.92, CI 95%: 0.81–0.97). No significant difference was observed on HMD_{CW} during T1 and T2: HMD_{CW} was 548 [314–1429] m for T1 and 677 [299–1552] m for T2 (Wilcoxon paired test analysis, $P = 0.351$). CV for HMD_{CW} was 29.5% [21.7–45.8]. Similarly, no significant difference ($P = 0.145$) was found between mean walking speeds at T1 (3.21 [2.78–3.97] km/h) and at T2 (3.00 [2.72–3.76] km/h). The ICC was high (0.94, CI 95%: 0.85–0.97) and CV was 5.4% [4.1–8.0]. The stop duration was the less reliable parameter with a median value of 1.42 [0.77–2.90] min for T1 and 1.58 [0.87–2.39] min for T2 ($P = 0.647$). CV for stop duration was 94.8% [64.9–171.7] and ICC was 0.46 [0.00–0.75].

Discussion: This study shows that the reliability of HMD_{CW} and of walking speed in the community was satisfactory. Conversely the mean stop duration was highly reliable. ICC of HMD_{CW} was in the high range of those usually observed in test-retest recording of maximal walking distance on treadmill in PAD patients.

Keywords: global positioning system, walking limitation, peripheral artery disease, exercise, maximal walking distance.

GROUPE SECTORIEL MIXTE PHYSIOLOGIE ET PHARMACOLOGIE RESPIRATOIRES: GSM1

CO-046
Adipokines differently modulate human lung macrophages polarization

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Rationale: Adipose tissue actively participates in inflammation and immunity, notably by the release of numerous factors including the adipokines. Macrophages can be committed to a continuum of functional phenotypes depending on various environmental cues. The two extremes of the spectrum are the classically activated macrophages by LPS (M1 macrophages) and the alternatively activated macrophages by IL-4/IL-13 (M2a macrophages). Adipokines could modulate modulate the polarization of monocyte-derived macrophages. In the present study, we explored the effect of some adipokines particularly involved in lung inflammation (adiponectin, leptin, visfatin and chemerin) on the polarization of human lung macrophages.

Material and methods: Lung macrophages were isolated from tissues obtained after surgical resection for cancer. Macrophages were treated with LPS (TLR4 agonist – 10 ng/mL), Poly I:C (TLR3 agonist −10 μg/mL) and IL-4 (10 ng/mL) in presence or not of Adiponectin (3–10–30 μg/mL), Leptin (1–10–100–1000 ng/mL), Chemerin (10–100–500–1000 ng/mL) and Visfatin (50–100–250–500 ng/mL). Cytokines were measured by ELISA in supernatants after 24 h culture. In addition adipokines were measured in supernatants of lung parenchymal explants and macrophages after 24 h culture.

Results: All adipokines were measured in lung parenchymal supernatants: basal concentrations were calculated at 4.32 μg/mL of interstitial tissue for adiponectin, 9.36 ng/mL for leptin, 517.5 ng/mL for chemerin and 164 ng/mL for visfatin. Only visfatin was expressed in macrophages supernatants. Leptin, only at the greatest concentration 1000 ng/mL, significantly induced the secretion of M1 cytokines (CCL3, CCL4, CCL5, CXCL8, IL6, TNFα) by unstimulated lung macrophages but had no effect on macrophages stimulated by LPS. On the contrary, adiponectin (APN) down-regulated LPS-induced and poly I:C-induced release of CCL2, CXCL10, CCL4 and CXCL1 (respectively $-73 \pm 11\%$, $-61.5 \pm 16\%$, $-56 \pm 7\%$ and $-68 \pm 8.5\%$ for LPS with APN 10 μg/mL and $-64.4 \pm 10\%$, $-78 \pm 8\%$, $-84 \pm 9\%$ and $-44.4 \pm 8.4\%$ for PolyI:C with APN 10 μg/mL). APN also decreased IL4-induced release of M2 cytokines: CCL13 ($-96 \pm 1\%$) and CCL18 ($-77.4 \pm 3.6\%$). Neither visfatin nor chemerin modulated cytokines release by lung macrophages with or without polarized stimulation.
Conclusion: Among these adipokines, only Adiponectin regulates cytokines release by human lung macrophages, at concentration close to that in lung parenchyma and blood.
Keywords: macrophages, obesity, adipokines, inflammation.

CO-047

The effects of Th1- or Th2-polarizing agents on the production of chemokines by lung epithelial cells

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Introduction: The respiratory tract's epithelial cells are prime targets for inhaled toxics and are actively involved in inflammatory and repair processes in the lung through the release of inflammatory mediators. The concept of cell polarization similar to the lymphocyte Th_1/Th_2 dichotomy was extended to other cell populations such as macrophages, natural killer or neutrophils. However, such a polarization in lung epithelial cells has been poorly explored, despite functional evidence demonstrating a distinct response of these cells when stimulated with Th_1-polarizing (LPS, TNF-α) or with Th_2-polarizing (IL-4, IL-13) agents. The aims of our study were to investigate the production of chemokines by lung epithelial cells cultured in the presence of Th_1- or Th_2-polarizing agents.
Methods: Primary human bronchial epithelial cells were isolated from patients undergoing surgery for lung carcinoma then stimulated for 24 hrs with LPS (1–1000 ng/mL), TNF-α (1–100 ng/mL), IL-4 (1–50 ng/mL) or IL-13 (1–50 ng/mL). Forty nine chemokines transcripts were assessed with RT-qPCR and Th_1-type (CCL20, CXCL1 and CXCL8) and Th_2-type (CCL26) chemokines were quantified in culture supernatants with ELISA.
Results: LPS increased CXCL14 transcript expression (4-fold) only, whereas exposure of cells to TNF-α increased the expression of CCL20 (17), CXCL8 (4) and CXCL14 (6) transcripts. Only the CCL26 transcripts were increased by more than 600-fold with IL-4. All the other 45 chemokine transcripts were unaffected in all conditions. At the protein level, TNF-α induced a rise in the production of CCL20, CXCL1 and CXCL8 when applied at 50 or 100 ng/mL. Maximal mean fold increases were 4.5, 1.4 and 2.4 respectively. On the other hand, LPS, IL-4 and IL-13 were devoid of effect on the production of these chemokines, whereas CCL26 was below the limit of detection in all conditions.
Conclusion: TNF-α was the main agonist able to impact the production of Th_1-type chemokines by primary bronchial epithelial cells. The effects of the other agonists known to induce the polarization of immune cells towards the Th_1 or Th_2 phenotype were limited in our experimental conditions. The concept of cell polarization may be more suitable for immune than for lung epithelial cells, with respect to chemokine production.
Keywords: lung epithelial cells, chemokines, polarization.

CO-048

Pharmacological CFTR inhibition decreased CD11b expression and phagocytosis capacity in human primary macrophages

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Early in life, cystic fibrosis (CF) patients are infected with microorganisms. Macrophages may however play a significant role in the initiating stages of the disease, *via* an inability to act as a suppressor cell. However, the role of macrophages has largely been underestimated in CF literature, whereas the focus being mostly on neutrophils and epithelial cells. Yet macrophage dysfunction may be the first step in cascade of events leading to chronic inflammation/infection in CF [1]. In order to highlight intrinsic CF macrophage defects, we have studied pharmacological CFTR inhibition on human primary macrophages functions: recognition patterns of invading pathogens, phagocytosis and cytokine/chimiokine productions.
Ficoll isolated adherent monocytes were differentiated into macrophages with GM-CSF (400 U/mL) during 6 days. The presence of CFTR in human primary macrophages is demonstrated by western blot. Cells were stimulated with $CFTR_{inh172}$ (10 μM, 72 h). We have focused to study some membrane markers of $CD71^+$ macrophages (mCD14, CD16, CD64, CD11b, TLR-2/4/5) by flow cytometry. Function of TLR-4/5 was studied by stimulation with their specific agonist, LPS (1–100 ng/mL) and flagellin (0.1–10 μg/mL). IL-1b, TNF-α and IL-8 supernatant levels were quantified by ELISA. Phagocytosis capacity was evaluated by two different methods. The first used heat-inactivated *Escherichia coli* linked to fluorescein. The second method was a microbiological assay with live *P. aeruginosa*.
Interestingly CFTR inhibition led to a significant decrease of CD11b expression as well as related phagocytosis but did not influence TLR-4/-5/-2, mCD14, CD16 and CD64 expressions as well as cytokine secretions.
Altogether less CD11b expression and decreased phagocytosis capacity should contribute to altered clearance of pathogens by macrophages. However alterations of macrophage functions seem thus not only to be associated with CFTR inhibition according to results observed on macrophages from CF patients [1].
Acknowledgments: Vaincre la Mucoviscidose and Biosit.
Reference:
1. Simonin-Le Jeune et al. PlosONE, 2013, 8(9):e75667.
Keywords: lung, macrophage, CFTR, inflammation.

CO-049

Characterization of novel cystic fibrosis mutations found in classical and infertile CF males in Indian population

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Cystic Fibrosis (CF) an autosomal recessive disorder caused by mutation in the CF Transmembrane conductance Regulator (CFTR) gene. CF is usually considered, as a very rare disease in Indian population. Whereas the mutations which are common to Caucasians are well characterized according to the degree of severity in CF disease, this is not the case in India. Most of the mutations identified in Indian population are rare and novel genetic counseling as well as adoption of therapeutic approach is particularly difficult when patient is presented with this rare variation. Recently we established a spectrum of mutation in CFTR gene from classical CF and from infertile male patients with CBAVD/CUAVD in Indian population. Among them S549N, L69H, G126S, Y852F, F87I, S118P, H139Q, F157C and F494L detected in patients with either Classical CF or infertility, were among rare missense mutations. In the present work we functionally studied these nine rare mutations by expressing GFP-CFTR constructs, and characterized them by western blot and automated iodide efflux assay via the screening platform BIOSCREEN. We determined CFTR maturation processing and CFTR channel activity. Seven of these mutations (G126S, Y852F, F87I, S118P, H139Q, F157C, F494L) present no apparent CFTR maturation defect. But two, S549N and L69H are considered as severe mutations. We show that substitution of Leucine to Histidine at 69 position of NH_2 terminal in CFTR domain leads to null maturation and abolished CFTR chloride channel activity like for the most common CF mutation F508del. Hence, F508del and L69H are categorized as class II defect. Interestingly, this defect is rescued with the investigational drug VX809. On the other hand, substitution of Serine with Asparagine at 549 position in NBD1 terminal domain of CFTR leads to diminished channel activity, this defect is rescued significantly with VX809, Miglustat, IsoLAB and lower temperature (27 °C). Thus S549N mutant can be categorized as II/III defect causing CFTR mutation. To conclude, this study identified in the indian CF population a novel class II mutation L69H and a classII/III mutation S549N that can be pharmacologically rescued.
Supported by the Charpak Fellowship programme 2012 of the French Embassy in India and Poitiers University.
Keywords: cystic fibrosis, Indian mutations, CFTR, pharmacology.

CO-050

Identifying neural drivers of the respiratory neural network in chronic obstructive pulmonary disease

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Introduction: Brainstem neural control of respiratory muscles have been recently shown to be altered in patients with chronic obstructive pulmonary disease (COPD) (PLOsONE 2013;8:e75740). The relationship between automatic and cortical network is still unknown in these patients despite its crucial role in the mechanisms preceding acute respiratory failure. We aimed in identifying causal interactions of the respiratory neural network during COPD at rest and during inspiratory resistive loading (IRL).
Methods: Fifteen COPD patients and 15 controls recruited from Bichat Hospital underwent cerebral fMRI (3T). Experimental protocol comprises firstly a resting state sequence during spontaneous breathing (SB) and secondly a block design paradigm during IRL to locate brain area involved in the respiratory muscles command. These target cerebral area were activated in both population. BOLD signal time series of 3 main brain regions of interest were then extracted from the left and right cortex (LC/RC) of the premotor and supplementary motor area (Brodmann area (BA) 6), from the brainstem (Lateral medulla-pons, LMP)). Partial Granger causality identifies neural drivers of the network in terms of causal density and flow of a node (directionality). It relies on linear multivariate autoregressive models. Assuming that $x(n)$ and $y(n)$ are the measured time courses of two brain regions (or voxels), Granger causality quantifies the usefulness of unique information in one of the time series in predicting values of the other. If incorporating past values of x improves the prediction of the current value of y, we say that x 'Granger causes' y.
Results: During SB in controls, the LMP cluster was accurately identified as being the neural driver of the network while the LC was a causal sink. In patients, the opposite was evidenced with a causal source shifted towards the left cortex. IRL balanced the relative role of the LMP and cortex clusters in controls, while in patients the LMP cluster was strongly becoming a causal sink.
Conclusions: These findings highlight profound alterations of the relationship between automatic and cortical network in COPD patients during SB and acute loading.
Fundings: BQR Université Paris 7, PHRC, Fondation du Souffle.
Keywords: respiratory neural network, brain, fMRI, Granger Causality, COPD.

CO-051

Effect of an acute exposure to etonogestrel, a progestin, on the central respiratory drive in mouse, involvement of medullary areas

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Several works have underlined the facilitatory influence of progesterone and progestins on the central respiratory drive (CRD) but the involved mechanism are not yet determined. The Congenital Central Alveolar Hypoventilation (CCHS) is a neuro-respiratory disease characterized by an alteration of the CO_2/H^+chemosensitivity due to dysfunction of the CO_2/H^+ chemosensitive/PHOX2b-positive neurons of the retrotrapezoid nucleus/parafacial respiratory group (RTN/pFRG).On the clinical level, CCHS is characterized by an important hypoventilation during sleep, which requires ventilatory support. In our laboratory, a recovery of the CO_2/H^+chemosensitivity was fortuitously observed in two CCHS women who took a potent synthetic progestin, the desogestrel (Straus et al., 2010).

The aim of the present work was to appreciate the effects of an acute exposure to etonogestrel (ETO), the main active metabolite of the desogestrel, on the CRD and to determine medullary areas that are involved.

Two experimental approaches were used: 1/an analysis of the effect of ETO (1 μM) on the CRD of *ex vivo* medullary-spinal cord preparations from newborn mice in both normopH and metabolic acidosis and 2/an analysis of the *c-fos* expression in medullary respiratory structures of *in vivo* juvenile mice receiving ETO *per os* (1 mg/kg) under both normocapnia and hypercapnia (8% CO_2).

The respiratory frequency was (*i*) increased in normopH by ETO in comparison to that measured on control preparations that were not exposed to ETO (≈+28%; $P = 0.058$) and (*ii*) unchanged by ETO under metabolic acidosis (≈+37% in preparations exposed to ETO, $P < 0.01$ and ≈+40% in control preparations not exposed to ETO, $P < 0.01$). The immunohistological detection of c-Fos revealed that ETO activated cells in some respiratory medullary areas *i.e.* the nucleus of the solitary tract and RTN/pFRG.

In conclusion, as a whole, data suggest that, by medullary mechanisms, the progestin ETO exerts a facilitatory influence on the CRD by acting at the level of respiratory structures. Besides, present data suggest, at least in newborn, that ETO do not potentiate the respiratory response to metabolic acidosis by medullary mechanisms.

Keywords: central respiratory drive, CO2 chemosensitivity, 'progesteronergic' systems.

GSM1 RESPIRATOIRE

CO-052

Oxygen capture in the human lung, a complete theory?

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Objective: To create a complete quantitative model of oxygen capture which takes care (i) of the dynamics of convection-diffusion in the acinus, (ii) of the dynamics of blood saturation and (iii) of their coupling.

Method: The problem can be solved by separating the gas and blood problem due to their different time scales, seconds for the respiratory cycle and, tenths of seconds for oxygen trapping by blood. On the gas side, the acinar spatio-temporal distribution of oxygen concentration and capture at rest and strong exercise are found through a numerical solution of the convection-diffusion-permeation equations in the moving acinus [1]. In the blood side study, we reformulate the Roughton and Forster mechanism but now in the time domain and obtain a totally different picture of capture, in agreement with the recent experiments of Tabuchi et al. [2]. This ensemble constitutes a complete quantitative theory of oxygen capture in terms of the parameters that govern human oxygen capture (namely, cardiac output, ventilation, hematocrit and etc.)

Results: The numerical solutions of these equations give the oxygen capture at rest and strong exercise in agreement with measured experimental values. In addition, we predict oxygen capture in various situations: (i) We give quantitative prediction about the role of ventilation-perfusion matching. (ii) We compute oxygen capture for high altitude and show that hyperventilation is necessary to maintain the metabolic rate. (iii) We show that, at sea level, we benefit from a respiratory reserve against mild edema and we give a prediction for the quantity of fluid in the lung above which edema has severe consequences on VO_2 and PAa.

Conclusion: The dynamics of oxygen extraction from the gas and capture by blood play a dominant role in respiration. The classical Bohr's and Roughton and Forster's steady state interpretation cannot provide a quantitative understanding of oxygen capture without the use of adjustable parameters.

References:

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2. Tabuchi, A. et al. Am. J. Respir. Crit. Care Med. (2013)188, 474–481.

Keywords: oxygen capture, hematocrit, edema, altitude, ventilation-perfusion.

CO-053

TRPV5 and TRPV6 implication in abnormal increase of constitutive Ca^{2+} influx in cystic fibrosis cells

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Several members of the transient receptor potential vanilloid (TRPV) channels family have already been described as emerging target for respiratory diseases such as asthma, and COPD (TRPV1, TRPV2). The genetic disease cystic fibrosis (CF) is caused by the mutation of the cystic fibrosis transmembrane conductance regulator (CFTR) gene and leads to defective airway epithelial sodium and chloride transports. The abnormal increase of calcium influx is a hallmark of CF cells. TRPV5 and TRPV6 display properties namely high Ca^{2+} selectivity and constitutive activity. The objective of this study is to investigate the involvement of TRPV5 and TRPV6 in abnormal increase of Ca^{2+} influx in CF cells.

16HBE14o-, CFBE41o- cell lines and freshly isolated human airway epithelial cells from CF (homozygotes F508del-CFTR) and non CF patients were used. The expression of TRPV5 and TRPV6 channels was verified by Western Blot and Ca^{2+} influx was measured using Fluo-4 acetoxymethyl ester probe.

TRPV5 and TRPV6 proteins are both expressed in these CF and non CF epithelial cells. Constitutive Ca^{2+} influx is 1.5 times higher in CF cells compared to non CF cells. This abnormal influx is normalized after F508del-CFTR rescued by 100 μM Miglustat 2 h or 27 °C for 48 h. Measurement of Ca^{2+} influx after applying the specific inhibitor of TRPV6, SOR-C27 (Bowen et al., 2013) showed a decrease of constitutive Ca^{2+} influx in non CF and more importantly in CF cells.

These results suggest that TRPV6 is implicated in the abnormal increase of constitutive Ca^{2+} influx in CF cells. Using iodide efflux and siRNA strategy, future experiments will investigate the impact of TRPV6 inhibition on CFTR activity in CF and non CF cells. These experiments will help to better understand the physical and/or functional interaction between Ca^{2+} transport proteins and CFTR protein.

Supported by the French association 'Vaincre la mucoviscidose'.

Keywords: calcium, human bronchial epithelial cells, cystic fibrosis, TRPV6, CFTR.

GROUPE SECTORIEL MIXTE PHYSIOLOGIE ET PHARMACOLOGIE CARDIOVASCULAIRES: GSM2

CO-054

Polycystin deficiency results in complete loss of nitric oxide release during sustained flow-mediated dilatation of conduit arteries in autosomal dominant polycystic kidney disease: possible reversal by dopamine

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Objectives: Autosomal dominant polycystic kidney disease (ADPKD) is a common renal hereditary disorder associated with increased cardiovascular mortality. ADPKD is due to mutations in PKD1 and PKD2 genes, encoding polycystin-1 and the calcium permeable nonselective cation channel polycystin-2 (TRPP2). These proteins form a plasma membrane complex, sensing flow variation and promoting calcium entry in the renal epithelium but also in vascular endothelium. Interestingly, dopaminergic type 5 receptor stimulation restored the endothelial mechanosensation and calcium-induced nitric oxide (NO) release in PKD1 knockout mice. However, the impact of polycystin deficiency, in ADPKD patients, on endothelium-derived factor release during flow variations in conduit arteries and the effect of dopamine stimulation are unknown.

Methods: In 21 normotensive ADPKD patients with normal kidney function and 21 healthy control subjects, endothelium-dependent flow-mediated dilatation was measured during a sustained hyperemic stimulation (hand skin heating) and during a transient stimulus (post-ischemic hyperemia). Local blood samples were drawn during heating to quantify plasma nitrite, indicator of NO availability, epoxyeicosatrienoic acids (EETs) and endothelin-1.

Results: Basal inflammatory and oxidative stress markers were similar between groups. Flow-mediated dilatation was lower in ADPKD patients than in controls during heating (16.1 ± 1.1 vs. 23.2 ± 1.0%, $P < 0.001$) but not during post-ischemic hyperemia (8.2 ± 0.4 vs. 8.2 ± 0.3%, $P = 0.99$), and without change in endothelium-independent dilatation to glyceryl trinitrate (27.8 ± 1.4 vs. 29.0 ± 1.6%, $P = 0.57$). Plasma nitrite increased during heating in controls but not in patients (30 ± 10 vs. −16 ± 8 nM, $P < 0.01$). Plasma EETs tended to increase in controls but not in patients without difference in endothelin-1 reduction. While the basal activity of the dopaminergic system was similar between groups, brachial infusion of dopamine (0.25–0.5 μg/kg/min) dose-dependently increased flow-mediated dilatation and restored the augmentation of nitrite during heating in ADPKD patients.

Conclusions: ADPKD patients display a loss of NO and subsequent reduction in endothelium-dependent dilatation of conduit arteries during sustained flow increase. The prevention of this alteration by dopamine may help to reduce the high prevalence of cardiovascular diseases in ADPKD.

Keywords: polycystin, endothelium, nitric oxide, dopamine.

CO-055

Functional microvascular rarefaction is reversed by simvastatin and lovastatin in the brain and skeletal muscle of spontaneously hypertensive rats

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Objective: Microvascular rarefaction is an aggravating factor of hypertensive end-organ damage. However, the microcirculatory effects of statins in hypertension remains unknown. Thus, this study was designed to investigate the acute effects of simvastatin and lovastatin on cerebral and muscular microcirculation in Spontaneously Hypertensive Rats (SHR)

Methods: Male normotensive Wistar rats (WKY) and SHR were divided into 4 groups of 6 animals each: WKY and SHR-CTL treated with 0.9% saline solution, and SHR + SIM and SHR + LOVA treated with simvastatin (SIM) and lovastatin (LOVA) 30 mg/kg/day during 3 days orally by gavage. We investigated brain and skeletal muscle (SM; gracilis muscle) Functional Capillary Density (FCD) using intravital fluorescence videomicroscopy after IV injection of fluorescein-isothiocyanate (FITC)-labeled dextran. All surgical procedures and protocols were approved in accordance with the internationally accepted principles for the Care and Use of Laboratory Animals (CEUA license # L-48/12).

Results: SIM administration reduced SBP in SHR (SHR-CTL 203 ± 3 vs. SHR + SIM 172 ± 6 mmHg; $P < 0.001$; in contrast LOVA treatment was not able to reduce SBP (SHR + LOVA 192 ± 3 mmHg). SHR showed a significantly lower FCD in the gracilis muscle compared to WKY (SHR-CTL 210 ± 17 vs. WKY 338 ± 16 capillaries/mm²; $P < 0.01$). SIM (SHR + SIM 447 ± 20 capillar-

ies/mm²) and LOVA (SHR + LOVA 418 ± 22 capillaries/mm²) treatment reverted functional capillary rarefaction in the SM of SHR (SHR-CTL 210 ± 17 capillaries/mm²; $P < 0.001$). Cerebral FCD was reduced in SHR compared with WKY (SHR-CTL 337 ± 61 vs. WKY 421 ± 35 capillaries/mm², $P < 0.05$). The administration of SIM (SHR + SIM 530 ± 31 capillaries/mm²) and LOVA (SHR + LOVA 471 ± 37 capillaries/mm²) during 3 days was capable to increase cerebral FCD in SHR (SHR-CTL 337 ± 61 capillaries/mm²; $P < 0.05$). Leukocyte rolling was significantly greater in SHR when compared with WKY (SHR-CTL 6.2 ± 0.7 vs. WKY 2.7 ± 0.5 cells/min; $P < 0.05$), and 3 day treatment with SIM (SHR + SIM 2.8 ± 0.6 cells/min; $P < 0.01$) and LOVA (SHR + LOVA 1.8 ± 0.5 cells/min; $P < 0.001$) reduced leukocyte rolling when compared with SHR (SHR-CTL 6.2 ± 0.7 cells/min).

Discussion: In addition to cholesterol-lowering effects, pleiotropic effects of statins could turn out to be a new therapeutic approach for improving microcirculatory function in hypertensive patients. However, it is necessary to further investigate the mechanisms and pathways which may additionally play important roles in statin-mediated cardiovascular protection in hypertension.

Keywords: microvascular rarefaction, spontaneously hypertensive rats, statins, pleiotropic effects.

CO-056
Withdrawn

CO-057
Pharmacological isolation of the pulmonary veins in the rat

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Objectives: In man, ablation of electrical connections between the pulmonary veins and the left atrium is a recognised treatment for paroxystic atrial fibrillation. The role of adrenergic stimulation on pulmonary veins (PVs) ectopy and atrial fibrillation initiation remain unclear. In the rat, left atrium (LA) and PVs cardiomyocytes have different reactions to adrenergic stimulation with a particular role for α-adrenergic receptor activation. We examine the effect of noradrenalin and the selective α-adrenergic agonist, cirazoline, on the conduction of electrical activity between the LA and the PVs of the rat.

Methods: An isolated right and left atria-PVs preparation from adult male Wistar rats was superfused with a Krebs-Henseleit solution at 37 °C. Cartography of conduction within the LA and along the PVs was recorded with a linear array of extracellular electrodes and the MC-Rack system. Dual intracellular microelectrode recording used a WPI Duo 773 electrometer amplifier.

Results: Under basal conditions with a sinusal rate of ~ 180 beats/min, the conduction of electrical activity was faster along the PVs than in LA. Noradrenalin (10^{-8}–10^{-6}M) increased conduction velocity only in the PVs.

Cirazoline (10^{-7}M) provoked a progressive loss of conduction in the PVs from the periphery (5 min) to the base of the vein (10 min) whereas conduction was maintained in LA. The α-adrenergic antagonist prazosin ($5*10^{-6}$M) led to the recovery of conduction along the PVs. This loss of electrical activity resulted from the depolarization of the diastolic membrane potential in the PVs whereas full over-shooting action potentials were still recorded in LA.

Conclusion: Selective activation of α-adrenergic receptors results in the functional isolation of the PVs in the rat.

This study was financed by a grant from 'Fondation Coeur et Recherche'.

Keywords: pulmonary veins, adrenergic stimulation.

CO-058
Deranged myofilament O'GlcNacylation and function in myocardium of obese patients

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The obesity-related cardiomyopathy is a poorly explained disease and yet is steadily increasing in developed countries. If abnormal myocardial energy metabolism has been demonstrated in obese patients, there is no data available on myofilaments sensitivity to calcium and post-translationnal modification or isoform shifts of sarcomeric proteins that could be involved in the contractile impairment

Therefore we conducted a study on obese and non obese human atrial trabeculae of the right atrium collected in the surgery room during cardiopulmonary bypass. We studied the contractile force of the trabeculae, the sensitivity of the myofilaments to Ca^{2+}. A transcriptom study was carried out. Western blots were performed in order to explore the post-translationnal modification of sarcomeric proteins including phosphorylation and O'GlcNacylation. Finally we explored the acute ex vivo effect of the modulation of O'GlcNac on the myofilament sensitivity to calcium.

We included 66 consecutive patients and performed the study on 61 of them. We were able to show that there was a significant contractile dysfunction in obese subjects (BMI > 30 kg/m^2) compared to normal subjects (BMI < 27.5 kg/m^2). This dysfunction was observed in both the force ($P = 0.01$) and myofilaments sensitivity to calcium concerning the Fmax ($P = 0.03$). No change in the expression of genes encoding sarcomeric proteins or enzymes involved in post-translationnal modification was observed. We found no phosphorylation modification of sarcomeric proteins like cMLC2 or cTnI that reflects respectively PKA and MLCK pathway in obese patients compared to normal-weight patients. Conversely, we showed in obese patients a decreasing O'GlcNacylation of proteins of 25 kDa ($P = 0.007$) and 130 kDa ($P = 0.03$) which include the sarcomeric proteins MLC2 and Troponin I. Finally, we pointed out that alteration of the O'GlcNac level by Azaserin altered the cardiac myofilaments sensitivity to calcium.

As far as we know, this is the first study that highlights before clinical and echocardiographic onset, such an association between impaired contractile function, an altered sensitivity to calcium, and a decreased O'GlcNacylation of possible sarcomeric proteins in humans.

Keywords: obesity related cardiomyopathy, O'GlcNac, contractile function.

CO-059
Trafficking defective mutations modulate Nav1.5 N glycosylation states.

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Aim: Brugada syndrome (BrS) is an inherited autosomal dominant cardiac channelopathy characterized by abnormal ECG pattern and an increased risk of sudden cardiac death. Several mutations on the cardiac sodium channel $Na_v1.5$ which are responsible for BrS lead to misfolded proteins that do not traffic properly to the plasma membrane and are instead retained in intracellular compartments. Although pharmacological rescue is commonly used to characterize misfolded mutants, underlying cellular retention mechanisms remain unclear. The aim of this work was to investigate trafficking defective $Na_v1.5$ mutants and their associated glycosylation states considering BrS patient heterozygosity and the presence of the β1-subunit.

Methods: This study combined cellular electrophysiological and biochemical approaches. Depending on the experiments, HEK293t cells were transiently transfected with either or both Wild Type (WT) and BrS mutations of $Na_v1.5$ and the β1-subunit.

Results: We show that BrS mutants exert a strong dominant negative effect upon WT sodium current density. Our data indicate that this effect requires the presence of the β1-subunit and is mediated by disruption of membrane trafficking of WT channels. Co-immunoprecipitation experiments demonstrate a physical interaction between mutant and WT α-subunits occurring only when the β1-subunit was present. Furthermore, we investigate the maturation pattern of membrane Na channels. Our data show distinct N-glycosylated states associated to different WT and mutant channels localization and function.

Discussion: This work highlights that β1-subunit and N-linked glycosylation process play key roles in modulating $Na_v1.5$ trafficking and function.

Keywords: voltage gated sodium channel, Nav1.5, glycosylation, trafficking defect, Brugada syndrome, heart.

GSM2 CARDIO-VASCULAIRE

CO-060
Vascular Nav channel regulation is involved in anti-anginal properties of ranolazine

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Background and aims: Ranolazine is a recently developed antianginal drug used for the treatment of patients with chronic stable angina. It is reported to be a selective inhibitor of the persistent sodium current (I_{NaP}), and to principally reduce the Na-dependent Ca^{2+} overload that occurs in cardiomyocytes during ischemia. Although vascular effects of ranolazine have been reported, its mechanism of action is uncertain. Na^+ channels (Na_v) present in arteries could be one target. Here, we have explored the effects of ranolazine on the rat aorta and described its molecular mechanisms of action.

Methods: Experiments were performed on rat aorta and cultured smooth muscle cells. We studied variations in the isometric tension of rat aortic rings in response to ranolazine in the presence of various vasoconstrictors and under conditions known to specifically activate smooth-muscle-cell I_{NaP}. Intracellular calcium variations were determined using the ratiometric fluorescent Ca^{2+} indicator Fura-2 on cultured smooth muscle cells obtained after enzymatic digestion of rat aorta.

Results: We observed that ranolazine induced vasodilatation and inhibited the vasoconstriction induced by veratridine an agonist of Na_v channel. These effects are independent of the presence of the endothelium. We have previously shown that slight depolarizations induced by low KCl concentrations activate I_{NaL}.Moreover, in the presence of prazosin (α-adrenergic antagonist) we selectively target smooth muscle I_{NaL} with KCl. We observed that ranolazine inhibited the contractile response induced by low KCl concentrations (below 15 mM). In the presence of prazosin, this inhibition persisted and was comparable to that of tetrodotoxin (1 μM) and to that of the selective inhibitor of Na/Ca exchanger (KB-R7943, 10 μM). The inhibition of smooth muscle Na_v channels was confirmed in cultured cells by fluorescent calcium imaging. We observed that ranolazine prevented the increase in intracellular calcium induced by veratridine. Furthermore, ranolazine inhibited the activity of TTX-sensitive voltage-gated Na_v channels both at sympathetic perivascular nerve terminals, through catecholamine release, and at the aortic myocyte level.

Conclusion: Ranolazine exerts a vasodilatory effect on rat aorta. This effect is in part attributable to I_{NaL} inhibition and may impact on the anti-anginal properties of the drug in addition to the cardiac mechanism.

Keywords: vasodilatation, artery, sodium channels.

CO-061
Regulation of TRPC and Orai1 channels by cardiac mineralocorticoid receptor: genomic effect of aldosterone

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Although aldosterone and mineralocorticoid receptor (MR) activation have been involved in the pathogenesis and progression of cardiac hypertrophy and heart failure, their cellular mechanism of action on cardiomyocyte function is not yet completely elucidated. Given that Ca^{2+} channels remodeling and aberrant Ca^{2+} fluxes have been described as a key step of cardiac MR deleterious effects, we hypothesized that TRPCs, Orai channels and their main activator STIM1 are directly regulated by aldosterone/MR signaling in cardiomyocytes.

Using a combination of molecular biological and biochemical approaches, we found that TRPC1, C3-6 isoforms and Orai1/STIM1 are expressed at transcript and protein levels in neonatal rat ventricular cardiomyocytes (NRVCs). Interestingly, mRNA expression of TRPC1, C5, and Orai1 were increased in ventricular cardiomyocytes treated for 24 h with 100 nM and 1 μM of aldosterone while TRPC3, C4, C6 and STIM1 were not affected. The selective MR antagonist, RU28318, prevented the aldosterone-enhanced TRPC1, C5 and Orai1 expression. Increased protein expression is also detectable for TRPC1 and STIM1 in treated cardiomyocytes. Additionally, stimulation of MR signaling by aldosterone during 24 h significantly enhanced SOC entry (measured by Fluo-4 and Fura-2 fluorescence) activated by Ca^{2+} stores depletion (with thapsigargin 2 μM plus caffeine 10 mM in presence of nifedipine) compared to untreated ventricular cardiomyocytes. The common TRPCs and Orai channels inhibitors, SKF-96365 40 μM and BTP2 5 μM, as well as the overexpression of the dominant-negative Orai1 or TRPC1 significantly reduced SOC entry, suggesting that TRPCs and/or Orai1 family are involved in the aldosterone-dependent Ca^{2+} response. Moreover, the modulation of SOCE appears to be mainly mediated by MR activation since MR blockade by RU28318 abolished the aldosterone-increased SOCE.
These findings showed that aldosterone/MR activation enhances TRPC1, C5 and Orai1/STIM1 expression and SOC entry in NRVCs, and that this may provide evidence for a novel pathway whereby Ca^{2+} entry and cardiac function are altered.
Keywords: TRPC channels, Orai channels, aldosterone, cardiomyocytes.

GS3 REIN

CO-062
Reduced connexin 43 expression protects mice against glomerulonephritis
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Glomerulonephritis (GN) includes a variety of renal pathologies which may lead to end-stage renal disease. We have recently observed an increased expression of the gap junctional protein connexin 43 (Cx43) within injured glomeruli of mice after induction of GN, by using the anti-glomerular basement membrane (aGBM) model of experimental nephropathy. Consequently, the aim of our work was to study the role of Cx43 in this model of GN. To this end, we compared the evolution of the disease between two different groups of mice, one normally expressing Cx43 (WT) and a second in which Cx43 expression was genetically reduced by half (Cx43+/−). Proteinuria, plasmatic urea and creatinine were significantly decreased in Cx43+/− mice 2 weeks after the neprhotoxic serum administration. Consequently, renal structure was highly improved as glomerular crescents, tubular dilation, monocyte infiltration and interstitial renal fibrosis were blunted in these mice. Colocalization experiments with nephrin and CD31/PECAM-1 indicated that Cx43 was mainly induced in podocytes and the glomerular capillaries. Interestingly, western blotting demonstrated that neprhin expression was preserved in Cx43+/− mice 2 weeks after the induction of GN, confirming the beneficial effect of Cx43 deletion in the integrity of glomerular podocytes during the progression of the disease. In addition, Cx43 expression was highly increased within damaged glomeruli in biopsies of patients suffering from different types of GN. Thus, our study demonstrates the deleterious role of Cx43 in GN and suggests that targeting Cx43 may protect against the progression of the disease.
Keywords: experimental nephropathy, aGBM, connexin 43.

CO-063
Does mineralocorticoid receptor antoganism could alleviate cyclosporin-induced nephrotoxicity in renal transplant recipients?
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Introduction: Cyclosporin-induced Nephrotoxicity (CIN) is a major adverse event but its pathophysiology remains unclear. Mineralocorticoid Receptor (MR) pharmacological antagonism prevents CIN in rats by modulating the expression of vasoactive factors (Perez-Rojas *et al.* AJPRP 2005). Our team has shown that MR is expressed in endothelial and vascular smooth muscle cells (VSMC) (Nguyen Dinh Cat *et al.* FASEB J 2010). Therefore, genetic MR manipulations in enthothelium (Nguyen Dinh Cat et al. FASEB J 2010) or VSMC (McCurley et al. NatMed 2012; Galmiche et al. Hypertension 2013) modify vascular function. Our working hypothesis is that vascular MR activation plays a key role in CIN.
Methods: Female mice with low-salt diet were used: (i) pharmacological approach: control, Ctrl (vehicle), CsA (CsA 100 mg/kg/d) and CsA + Can (CsA + canrenoate 30 mg/kg/d, MR antagonist); (ii) genetic approach: conditional MR knock-out in VSMC (MRKO-VSMC) or in Endothelial Cells (MRKO-EC) treated or not with CsA 100 mg/kg/d.
Results: Body weight loss is greater in cyclosporin-treated groups ($P < 0.05$). Renal function is impaired ($P < 0.05$) and cyclosporin induces renal histological damages that are prevented by MR antagonism or by MR KO in VSMC but not in endothelial cells. Canrenoate and MR KO in VSMC also prevent cyclosporin-induced renal expression (mRNA) of NGAL (Neutrophil Gelatinase Associated Lipocalin), a kidney damage marker. Cyclosporin induced NGAL expression in proximal tubules (immunohistochemistry); this effect is prevented by MR antagonism and MR KO in VSMC but not in endothelial cells.
Conclusions: We show that MR antagonism has beneficial effect on cyclosporin-induced renal damages that, at least partially, involve VSMC MR. The underlying cellular mechanisms are currently under investigation. A clinical trial testing the safety of MR antagonism (eplerenone) in renal transplant recipients treated with cyclosporin is currently ongoing.
Keywords: nephrotoxicity, cyclosporin, mineralocorticoid receptor, vascular smooth muscle cells.

CO-064
Mechanism of acidosis-induced adaptive proliferation of collecting duct intercalated cells
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Background: Kidneys adapt to acid load by increasing proton secretion by collecting duct intercalated cells (AIC). This adaptation stems in part from increased number of AICs. We reported previously that acid loading induces proliferation of AICs in mouse outer medullary collecting duct (OMCD) and that this process depends on the production of growth and differentiation factor 15 (Gdf15), a member of TGFβ super family (JASN, 19:1965–1974, 2008). Here we investigated the mechanisms of Gdf15 production and its involvement in mitosis orientation.
Methods: Acidosis was induced by feeding mice a NH_4Cl-enriched diet. OMCDs were microdissected from collagenase-treated kidneys. mRNAs were quantified by RT-quantitative PCR, number of AICs was counted after immunolabeling with an AE1 antibody. AICs proliferation was evaluated by the percentage of AE1 positive cells and the number of doublets of AE1 positive cells. The angle of AICs doublets with the axis of the tubule was determined after confocal imaging of isolated OMCDs and 3D reconstruction.
Results: Within 2 days, acidosis induced a 3-fold over expression of p53. NH_4Cl-induced over expression of Gdf15 and cyclin D1 were abolished in $p53^{-/-}$ mice which, accordingly, developed stronger acidosis. Because a) activation of proton secretion is expected to decrease ATP level and b) stimulation of AMPK activates p53 in many systems, we looked at the involvement of AMPK in AICs proliferation. Acid loading increased neither proliferation of AICs nor Gdf15 expression in OMCD from α_1 or α_2 $AMPK^{-/-}$ mice. These mice also displayed deeper acidosis than WT mice. In control mice, the angle between the axis of AIC doublets and the tubule axis was evenly distributed from 0 to 90°. Conversely, in acidotic mice, the number of doublets increased and they were preferentially oriented in parallel with the tubule axis.
Conclusions: Proliferation of AICS participates in the renal adaptation that maintains acid base balance in response to acid loading. This adaptive proliferation is dependent on activation of AMPK kinase and p53 over expression. Gdf15 triggers axial proliferation of AICs.
Keywords: intercalated cells, proliferation, p53, Gdf15, metabolic acidosis.

CO-065
Endoplasmic reticulum stress drives proteinuria-induced kidney lesions via Lipocalin2
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Objective: Proteinuria is an essential prognosis factor in chronic kidney diseases (CKD) progression in humans, and may have a direct role in tubulointerstitial lesions development, a critical element of CKD progression. The aim of this study was to decipher the mechanisms involved in proteinuria-induced tubulointerstitial injury.
Methods: Different models of proteinuria (genetic or pharmacologically-induced) applied to several transgenic models were studied. Endoplasmic reticulum (ER) stress inductors or inhibitors were also evaluated in these mice. In vitro, studies used different cell lines (IMCD cells, primary cultured renal cells, mouse embryonic fibroblasts) exposed to albumin or ER stress modulators. ER stress markers were evaluated by western blot, immunohistochemistry and qRT-PCR. Apoptosis was evaluated by TUNEL, FACS and western blot.
Results: Studying different genetic and pharmacologic models of proteinuria, we observed that proteinuria induced ER stress in tubular cells in a calcium-dependant manner. Furthermore, we established that ER stress was not only a marker but a mediator of CKD progression: inhibition of ER stress with phenylbutyrate, a drug already used in humans, reduced tubular lesions and renal dysfunction in proteinuric mice. More importantly, we identified a novel activation pathway of CKD progression. In fact, we demonstrated that proteinuria-induced ER stress regulates Lipocalin2 (Lcn2) expression, a small iron binding protein implicated in CKD progression. These results were not restricted to animal models, since we identified similar Lcn2 activation in tubular cells of proteinuric patients. By using activating transcription factor 4 (ATF4)-null cells, we demonstrated that ATF4 was crucially involved in Lcn2 overexpression during albumin and ER stress exposure. Moreover, by using double transgenic mice, we demonstrated Lcn2 drove apoptosis due to ER stress and proteinuria in tubular cells. Finally, we demonstrated that Lcn2 invalidation protected proteinuric mice from tubulo-interstitial lesions and, more importantly, from long-term mortality.
Discussion: This work identifies a novel pathway implicating ER stress in tubulo-interstitial lesions and apoptosis of murine proteinuric nephropathies, by showing the critical role of the ATF4/Lcn2 axis. Moreover, these data suggest that ER stress, which may be inhibited with phenylbutyrate, is a new therapeutic target in proteinuric CKD progression.
Keywords: chronic kidney disease, endoplasmic reticulum stress, proteinuria, lipocalin2, phenylbutyrate.

CO-066

Cystatin c based GFR equation does not outperform creatinine base formulas in obese CKD patients

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Introduction: Recent findings suggest that cystatin C is independently associated with obesity, body mass index and waist circumference. Hence, cystatin C based equation could overestimate the chronic kidney disease (CKD) prevalence for overweight and obese patients. The objective of this study was to assess the relation of cystatin C and body composition in overweight and obese patient with CKD and evaluate the accuracy of cystatin C based equation for glomerular filtration rate (GFR) estimation.

Materials and methods: This prospective study included 90 patients with BMI > 25 kg/m^2 who performed a gold standard clearance by inulin or iohexol (mGFR). Patients were separated in two groups, one group with a GFR < 60 mL/min/1.73 m^2 and a second group with GFR ≥ 60 mL/min/1.73 m^2. All patients underwent bioelectrical impedancemetry for body composition analysis and cystatin C measurement. GFR was also estimated by equations derived from creatinine ($eGFR_{CKD\text{-}EPI}$) or cystatin ($eGFR_{cyst}$) or both ($eGFR_{cyst\text{-}creat}$). Correlations were calculated with pearson correlation. Mean Bias (mGFR – eGFR) and accuracy within 30% were calculated for the three equations.

Results: Cystatin c was positively correlated with fat mass ($r = 0.35$, $P = 0.04$) and waist circumference ($r = 0.37$; $P = 0.04$) only in the GFR ≥ 60 mL/min/1.73 m^2 group, while no relation was found in the group with GFR < 60 mL/min/1.73 m^2. In the group with GFR < 60 mL/min/1.73 m^2, mGFR was 40.6 ± 1.5, $eGFR_{CKD\text{-}EPI}$ was 44.8 ± 1.9, $eGFR_{cyst}$ was 43.7 ± 2.6 and $eGFR_{cyst\text{-}creat}$ was 43.7 ± 2.1 mL/min/1.73 m^2. $eGFR_{CKD\text{-}EPI}$ has less bias than $eGFR_{cyst}$ and $eGFR_{cyst\text{-}creat}$ (2.1. ± 4 vs. 8 ± 1, $P = 0.05$ and 6 ± 0.7 mL/min/1.73 m^2, $P = 0.1$ respectively). The accuracy 30% was 87% vs. 75% and 90%, $P = 0.06$ and $P = 0.9$ for $eGFR_{CKDEPI}$, $eGFR_{cyst}$ and $eGFR_{cyst\text{-}creat}$ respectively.

Conclusion: Body fat is only a determinant of cystatin C in obese and overweight CKD patients with a GFR > 60 mL/min/1.73 m^2, but not in patient with GFR < 60 mL/min/1.73 m^2. $eGFR_{cyst}$ and $eGFR_{cyst\text{-}creat}$ could be used as an accurate estimation of GFR, but do not outperformed $eGFR_{CKDEPI}$.

Keywords: obese, GFR, cystatine C, fat mass, bioimpedancemetry.

CO-067

Height-independent GFR predicting equation in children: a possible routine screening tool for kidney disease in laboratories

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Objectives: According to KDOQI recommendations, determination of plasma creatinine (Pcr) should be associated to an estimation of GFR (eGFR). However, in the pediatric population, eGFR is rarely determined by laboratories as Schwartz formula requires height information, which is normally not available in clinical laboratory databases. Pottel et al. [1] developed a height-independent equation eDFG = 107.3/(Pcr/Q) with Q = median of Pcr for each age class. The aim of the study was (i) to adapt this equation to our laboratory (Pottel-Lyon) and (ii) to compare its performance to the original-Pottel equation and to the Schwartz equation.

Methods: (i) All first Pcr determination performed in children (1–18 years old) in the pediatric hospital in 2010 and 2011 were collected. Median of Pcr was determined for sex and each 1-year-age-interval and considered as the locally adapted coefficient (Q-Lyon). (ii) Among the whole population, 438 true measurements of GFR (mGFR, inulin or iohexol clearance, 359 children) were performed and compared to eGFR calculated by (i) the Pottel-Lyon, (ii) the original-Pottel equation and (iii) the Schwartz equation. Pcr was determined by an IDMS-standardized enzymatic assay.

Results: The local coefficient Q-Lyon was determined with the first Pcr measurement of 12219 children (53% males, mean Pcr = 43 ± 44 [12–2122] µM). Children with a mGFR (58% males) were aged of 10 ± 3 years (3–18) with a mean Pcr of 55 ± 28 (17–262) µM and a mean of eGFR of 93 ± 33 (18–204) mL/min/1.73 m^2. The Pottel-Lyon equation has a significant lower bias compared to the original-Pottel and to the Schwartz equation (0.9 ± 21.2, 8.9 ± 23.1 and 10.0 ± 22.5, respectively) and a better 30% accuracy (87.0% vs. 79.6% and 81.0%, respectively). The performance in identifying patients with renal dysfunction (GFR

Discussion and conclusion: The GFR-predicting-equations (Schwartz, original Pottel and adapted Pottel-Lyon formulas) are reliable with a better performance of the adapted Pottel-Lyon equation. When height information is not available, the height-independent Pottel equation can be used by medical analysis laboratories as an excellent screening tool for kidney disease.

Reference:
1. Pottel H, Pediatr Nephrol 2012 and Hoste L, NDT 2013.

Keywords: GFR-predicting-equations, children, height-independent GFR-predicting equation, Schwartz equation.

CO-068

Functional characterization of a new sodium channel implicated in renal sodium reabsorption

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Renal sodium retention in nephrotic syndrome results from an impairment of Na^+ reabsorption in the cortical collecting duct (CCD). The traditional concept of Na^+ retention attributes Na^+ reabsorption in the CCD to the renin-angiotensin-aldosterone system (RAAS). Previous studies have revealed that, during nephrotic syndrome, Na^+ reabsorption was independent of RAAS and that ENaC (Epithelial Na^+ channel) was not implicated [1]. A new Na^+ channel independent of RAAS system and sensitive to amiloride has recently been identified in the CCD in the nephrotic syndrome: a short isoform of ASIC2b (unpublished data). It is the first time that expression of an ASIC channel has been detected in the kidney. ASICs are proton-gated channels, members of the degenerin (DEG)/epithelial sodium (ENaC) superfamily, expressed principally in the central nervous system and in peripheral sensory neurons. ASIC2b is a subtype of ASIC2, it does not form a H^+-gated channel by itself but may associate with other subunits to form a functional channel. Here we report, for the first time, the functional characterization of this new isoform of ASIC2b, after heterologous expression in *Xenopus laevis* oocytes. Amiloride sensitive currents were recorded in oocytes expressing different combination of ASIC2 and ENaC subunits, using the two-electrode voltage-clamp technique. When co-expressed with αENaC and βENaC or with αENaC and γENaC subunits, ASIC2b can form amiloride-sensitive Na^+ channels. We also analyzed the electrophysiological properties of the heteromeric ASIC2a/ASIC2b complex and we show that ASIC2b attenuates the desensitization process of the ASIC2a channel. These data identify ASIC2b as a new regulatory subunit and suggest that it could be implicated in Na^+ reabsorption in the CCD during nephrotic syndrome.

Reference: 1. Ackermann D, Mordasini D, Cheval L, Imbert-Teboul M, Vogt B, Doucet A. Sodium retention and ascites formation in a cholestatic mice model: role of aldosterone and mineralocorticoid receptor? Hepatology. 2007; 46:173–9.

Keywords: sodium retention, collecting duct, acid-sensing ion channel (ASIC), epithelial sodium channels (ENaC).

CO-069

Dynamic changes in Barttin phosphorylation regulate ClC-K1 channel activity

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Sodium chloride (NaCl) transport in the kidney plays a major role in blood pressure regulation. ClC-K chloride channels from the ClC channels family, are necessary for NaCl reabsorption in the distal nephron. Subcellular localization and function of ClC-K channels are regulated by Barttin, an accessory subunit. Barttin improves the stability of ClC-K proteins and promotes their insertion into the plasma membrane. The importance of ClC-K/barttin channels is highlighted by several genetic diseases. Mutations in ClC-K channels or Barttin result in two forms of Bartter syndrome, a salt-losing renal tubulopathy. However, the molecular mechanisms that account for the regulation of chloride transport by ClC-K/Barttin channels remain unknown.

By a semi quantitative phospho-proteomic approach on isolated distal nephron, we identified an increase in phosphorylation of Barttin on a serine in mice fed with a low NaCl diet (0% Na^+) when compare to distal nephron isolated from mice fed a normal (0.3% Na^+) NaCl diet. Since chloride absorption by the distal nephron of mice fed a low NaCl diet is increased, we investigated the role of this phosphorylation site in the regulation of chloride transport by the Barttin/ClC-K channels complex.

Barttin cDNA was mutated *in vitro* to generate two mutants of the phosphorylation site: a non-phosphorylated (Serine to Alanine) form and a phosphomimic form (Serine to Glutamate). ClC-K1 was expressed in *Xenopus laevis* oocytes along with the wild-type form or the different mutant forms of Barttin and currents were recorded by two-electrode voltage-clamp. We observed that ClC-K1 currents were increased almost 2 folds in oocytes expressing the phosphomimic form of Barttin when compared to wild-type Barttin. By contrast, ClC-K1 currents were decreased by 30% in oocytes expressing the non-phosphorylated form of Barttin.

All together our results indicate that dynamic changes in Barttin phosphorylation modulate ClC-K channel activity and hence are involved in the mechanisms of adaptation of renal chloride absorption in response to changes in dietary NaCl intake.

Keywords: renal chloride transport, regulation.

GROUPE SECTORIEL MIXTE NEUROPHYSIOLOGIE ET NEUROPSYCHOPHARMACOLOGIE: GSM7

CO-070
Impact of rt-PA in serum BDNF levels in stroke patients

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Brain-derived neurotrophic factor (BDNF) is a neurotrophin critical for many brain functions. In the circulating compartment, low serum BDNF has been recently associated with increased risk of stroke incidence by the Framingham Study. BDNF is synthesized as a precursor protein that is processed extracellularly by matrix metalloproteinase or plasmin into the mature form. In the other hand, rt-PA is the only approved treatment of thromboembolic stroke through activation of plasminogen into plasmin that dissolves blood clots. In this context, the aim of our study was to test the hypothesis that rt-PA could, above of its well known fibrinolytic action, also have an impact on the level of serum BDNF in stroke.

Therefore, a single-center prospective longitudinal study was conducted including patients hospitalized for ischemic stroke within the neurovascular intensive care unit of the University hospital of Dijon. In this study, 12 stroke patients non-treated and 23 stroke patients treated with rt-PA were included and underwent 4 blood samples (at the inclusion and at day 1, 7 and 90). Using ELISA tests, BDNF levels were measured in blood serum and in addition, the number of platelets was also assessed.

Our results showed a significant increase in serum BDNF levels at D1 and D7 in rt-PA-treated patients as compared to non-treated patients with no correlation with the number of platelets. Taken together, our data suggest that rt-PA has a impact in serum BDNF levels in stroke patients.

Keywords: brain-derived neurotrophic factor, stroke, rt-PA, serum.

CO-071
Pleiotropic strategies in ischemic stroke: impact of a pharmacological modulation by atorvastatin

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Ischemic stroke is still a heavy public health burden, as effective therapeutic means remain scarce. Atorvastatin (AT), widely used as a preventive treatment in the cardiovascular field, possesses pleiotropic effects that confer to it protective properties in ischemic stroke. The aims of this work were to assess and characterize the neuro- and vasculoprotective effects of AT in cerebral ischemia, and the part played by PPARα.

Wild-type and PPARα-KO C57BL6/J mice were submitted to cerebral ischemia-reperfusion (I/R) and treated with AT before I/R for 14 days (AT10 group: 10 mg/kg/d) or acutely after I/R for 24 or 72 h (AT10 group: 10 mg/kg/d and AT20 group: 20 mg/kg/d). Neuroprotection was assessed histologically and by a sensorimotor evaluation. Vasculoprotection was assessed on the middle cerebral artery (MCA) itself by ex-vivo vascular reactivity in response to increasing doses of carbamoylcholine, an analogue of acetylcholine, to appreciate the endothelial function. Type-IV collagen was studied by immunofluorescence as a marker of the integrity of cerebral microvessels. The adhesion protein ICAM-1 and the enzyme myeloperoxydase, specific for neutrophils, were studied by immunohistochemistry to assess the interactions between leukocytes and the vascular wall.

Preventive and acute treatments with AT induced a reduction in lesion volumes, functional recovery was markedly improved in acutely treated animals (mean neurological score after 72 h: AT20 = 1.07 ± 0.96 vs. AT10 = 2.29 ± 1.14; $P = 0.016$; vehicle 2.33 ± 0.90; $P = 0.007$). The acute treatment with AT helped to preserve the endothelial function, the response and the sensitivity to carbamoylcholine being improved in AT20-treated mice. Microvessels were also protected; their density and integrity were significantly higher after 72 h of reperfusion. Treatment with atorvastatin also reduced the interactions between leukocytes and the endothelium. The expression of ICAM-1 and the number of infiltrated neutrophils were significantly reduced in treated animals after 24 h of reperfusion. Finally, it appears that PPARα is necessary for AT neuro- and vasculoprotective effects to take place, as PPARα-KO mice were not sensitive to the protective effects of atorvastatin.

Atorvastatin is protective in ischemic stroke, preventively as acutely. This work highlights the interest of atorvastatin as a pleiotropic neuroprotective agent in ischemic stroke.

Keywords: ischemic stroke, pleiotropic strategies, atorvastatin.

CO-072
Activation of TREK-1 by morphine results in analgesia without adverse side-effects

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Morphine, the gold standard pain reliever for severe acute or chronic pain, suffers from invalidating adverse effects which can alter quality of life of patients and in some rare cases jeopardize the vital prognosis. Unfortunately, morphine elicits both therapeutic and adverse effects, primarily through the same μ opioid receptor subtype, which makes it difficult to separate the two types of effects. We found that the TREK-1 potassium channel is positively modulated following μOR activation by morphine which suggests its possible involvement in the effects of μOR agonists like morphine. Consequently, we evaluated the implication of these K^+ channel in the analgesic effects and main adverse effects of morphine using TREK-1 knock-out mice and their wild-type littermates. We demonstrated for the first time that (i) there is a major involvement of the TREK-1 channel in the analgesic effect of μOR agonists both in acute nociceptive and painful pathological conditions and that (ii) this channel is not involved in the usual adverse effects of acute or chronic exposure to morphine such as constipation, respiratory depression and dependence. Beneficial and deleterious effects of morphine are thus mediated through different signaling pathways downstream from μ opioid receptor. These observations suggest that direct activation of the TREK-1 channel, acting downstream from the μ opioid receptor, might have strong analgesic effects without opioids-like adverse effects.

Keywords: morphine, analgesic effect, adverse side effect, ionic channels, TREK-1.

CO-073
Serotonin 5-HT6 receptors blockade and memory performances: new data suggesting a glutamatergic mediation

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Introduction: 5-HT_6 receptors (5-HT_6R) are expressed in brain regions associated with learning and memory. It has been shown that pharmacological blockade of 5-HT_6R improves memory performances in rodents but the underlying mechanisms are still misunderstood. One hypothesis is that 5-HT_6R antagonists increase the release of acetylcholine and glutamate. We investigated here the involvement of the glutamatergic system in the mnesic effects of 5-HT_6R blockade.

Method: Glutamatergic transmission was modulated through i) the competitive and reversible blockade of NMDA receptors (NMDAR) with the antagonist CGS 19755 (CGS), and ii) the potentiation of the glutamate-induced NMDAR activation using the glycine transporter type 1 (GlyT1) inhibitor NFPS. A selective 5-HT_6R antagonist, SB-271046 (SB, 10 mg/kg), was administered either alone or in combination with selected sub-active doses of CGS (3 mg/kg) or NFPS (0.625 mg/kg), in 3-month male NMRI mice. Spatial recognition memory performances were assessed in the two-trial place recognition task in a Y-maze (inter-trial interval ITI of 2 h or 4 h) [1].

Results: For a 4 h ITI, CGS tended to impair recognition performances, and this was counterbalanced by SB [discrimination index di: +73% vs. CGS]. In contrast, for a 2 h ITI, the association of CGS with SB significantly impaired recognition performances [di: −72% vs. SB]. Mice treated with NFPS were able to discriminate the novel arm after both ITI. Moreover, association of NFPS with SB tended to improve recognition performances after both ITI [di: 2 h ITI: +59% and 4 h ITI: +67% vs. SB].

Discussion: Our data support the idea that the effects of the 5-HT_6R blockade are mediated by a glutamatergic modulation. Indeed, i) NMDAR blockade by CGS impaired the SB-elicited memory performances (2 h ITI) though CGS had no direct deleterious effects and ii) NFPS-elicited NMDAR potentiation tended to improve recognition memory performances when co-administered with the 5-HT_6R antagonist. Such a new knowledge on the mechanisms involved in the mnesic effects of 5-HT_6R blockade opens new perspectives for the treatment of memory disorders associated to aging, neurodegenerative, or psychiatric diseases.

Reference: 1. Dellu et al., 1992. Brain Res., 588: 132–139.

Keywords: memory and cognitive disorders, serotonin, glutamate.

CO-074
Pharmacological manipulations of striatal interneurons induce a phenotype of dystonia in the monkey

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Objective: Dystonia is defined as a syndrome of sustained muscular contraction leading to repetitive movements and abnormal postures. To date, its pathophysiology remains unclear. A line of evidence suggests that the cholinergic (Ach-I) and GABAergic (GABA-I) interneurons of the striatum may play a critical role in its pathophysiology. Indeed, cholinergic antagonists are the only pharmacological drugs effective in the treatment of dystonia and acetylcholine applied on striatal brain slides corrects the abnormal cortico-striatal plasticity observed in transgenic mice exhibiting the mutant form of the torsinA. Furthermore, blocking GABA-I in rodents induce dystonic-like movements. Taken as a whole, this data suggests that increasing intrastriatal acetylcholine and decreasing GABAergic transmission may induce a phenotype of dystonia. Our aim was to test this hypothesis in non-human primates.

Methods: Intra-striatal (AC-2 mm, L + 12) microinjections of 2–8 μl Oxotremorine (Oxo, Ach-agonist), 0.5–4 μl Bicucculline (Bic, $GABA_A$ antagonist) or saline (Nacl 9‰) were performed in two macaca fascicularis under neuronavigation control. Animal behavior was studied in a primate chair before and for 2 h following injections. It was then blindly assessed on videos for clinical symptoms using an adapted form of the dystonia BFM rating scale.

Results: Abnormal involontary movements (AIMs) associating both tonic dystonic postures and myoclonic jerks were observed in the hemibody contralateral to injections whereas saline injections had no effect. These AIMs affected distal parts of the limbs, the trunk and sometimes the face and led at the highest volume to focal epileptic seizures. Bic injections had a quicker but shorter effect whereas Oxo injections had a delayed but prolonged effect.

Discussion: These preliminary results suggest that modifying interneuron activity within the striatum do indeed induce a phenotype of dystonia. They also reveal the dramatic effect of the striatal network on cortical excitability.

Keywords: dystonia, pathophysiology, striatal interneurons, primate model, acetylcholine, GABA.

CO-075

Effect of hypertension and physical training on cerebral endothelial BDNF expression

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Introduction: While the neuron is generally considered as the unique source of brain-derived neurotrophic factor (BDNF), we recently reported high BDNF expression in endothelial cells of peripheral vessels [1]. This expression was increased by physical training and decreased by hypertension, that are known to improve and alter endothelial function, respectively. Besides there are an increasing number of studies in favor of a link between cerebral endothelial dysfunction and cognitive decline. Whereas BDNF is largely involved in cognition, studies aimed at explaining the link between cerebral endothelial BDNF and endothelial dysfunction are lacking.

Aim of the study: To explore the effect of hypertension and physical training on BDNF expression in endothelial cells from intracerebral vessels.

Methods: Endothelial BDNF localization (immunostaining) in the hippocampus, striatum and cortex was assessed on brain slices collected from spontaneously hypertensive (SHR) and normal Wistar Kyoto (WKY) rats which were subjected or not to physical training. Rats assigned to physical training were trained on a rodent treadmill 30 min/day, 18 m/min for 7 consecutive days. Systolic and diastolic blood pressure and heart rate were measured before and after training by using the indirect tail-cuff method.

Results: Hypertension decreased endothelial BDNF expression in all the structures. In WKY, physical training increased endothelial BDNF expression in the cortex and hippocampus but not in the striatum. In SHR, physical training improved endothelial BDNF expression in all the structures. In the cortex, endothelial BDNF expression recovered control value. In SHR as WKY, physical training did not change blood pressure and heart rate.

Conclusion: Our results suggest endothelium BDNF expression by intracerebral vessels as a marker of cerebral endothelial function. As cognitive function is improved by physical training and altered in hypertension, our study open new perspectives on the role of cerebral endothelium-derived BDNF in cognition.

Reference: 1. Prigent-Tessier et al. Physical training and hypertension have opposite effects on endothelial brain-derived neurotrophic factor expression. Cardiovascular research. 2013;100:374–382.

Keywords: BDNF, endothelial cells, hypertension, physical training.

GSM7 NEUROLOGIE

CO-076

Cortical voice processing in cochlear-implanted children: an electrophysiological study

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Objective: In children with prelingual deafness, the use of cochlear implants can restore both auditory input to the auditory cortex and the ability to acquire spoken language. Language development is strongly intertwined with voice perception. The aim of this electrophysiological study was to investigate human voice processing with cortical auditory evoked potentials (AEPs) in cochlear-implanted (CI) children.

Material and method: Eight CI children (2 males, 6 females) aged 4–12 years (mean age: 8 years), fitted with a unilateral cochlear implant (CI group) for congenital sensorineural hearing loss were included in this study. All of them had good auditory and language performance. They were investigated with cortical AEPs and compared with 8 normal-hearing age-matched controls (mean: 8.5 year-old). The electroencephalogram were recording from 28 Ag-AgCl cup electrodes. The auditory stimuli were vocal and non-vocal sounds delivered in free field. The vocal non-speech sounds were produced by a large number of speakers of both genders and different ages. Non-vocal sounds consisted of sounds from a wide variety of sources, including human environments, musical instruments, and nature. Independent component analysis was used to minimize the cochlear implant artifact in cortical AEPs.

Results: Fronto-temporal positivity to voice was found in normal-hearing children with a significant effect in the 140–240 ms latency range. In the CI children group, we found a positive response to voice in the 170–250 ms latency range with a more diffuse and anterior distribution than in the normal-hearing children.Fronto-central responses (P1-N2-N4 waves) did not differ between the 2 groups.

Discussion/Conclusion: Response to voice was recorded in normal-hearing and CI children. The topography and latency of response to voice differed from that recorded in normal-hearing children. This finding argued for cortical voice processing reorganization in congenitally deaf children fitted with a cochlear implant.

Keywords: cortical auditory evoked potentials, cochlear implant, voice processing, children.

CO-077

Diabetic microangiopathy: implication for stroke severity and delayed angiogenesis after permanent cerebral occlusion in mice

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Objectives: Diabetes increases stroke mortality and delays recovery. Pre-existing associated microvascular disease could explain increased severity but this has been poorly investigated. The aim of our study was to characterize microvasculature in type 1-diabetic mice and consequences on angiogenesis, important in brain repair.

Material and methods: Type-1 diabetes was induced in C57Bl6J mice by streptozotocin injections. After 8 weeks of diabetes, neurovascular network was evaluated by (i) cerebral vasoreactivity to inhaled CO2 and NO donor, (ii) protein expression of eNOS and nNOS, (iii) capillary diameter by CD31+ immunohistochemistry, (iv) expression of angiogenic (VEGFa, Ang2) and vessel maturation (Ang1, TGFβ and PDGFβ) factors and of blood brain barrier (BBB) (claudin-5, occludin, ZO-1 and Ve-Cadhérine) component by RT-PCR and Western Blot and at last (6) structural morphology of arterioles and capillaries by Transmission Electronic Microscopy (TEM). Consequences on permanent focal cerebral ischemia (pMCAo) in diabetic mice were evaluated by infarct volume (cresyl violet and MRI), neurological deficit at D1 and D7, neuronal death (Tunel+/NeuN+ cells) and BBB permeability (Evans blue extravasation) at D1. Angiogenesis was evaluated by CD31+/Ki67+ cells double immunostaining and evolution of angiogenic and vessel maturation factors (RT-PCR and Western Blot) and of BBB protein expression mRNA (RT-PCR), at D1 and D7.

Results: In diabetic mice: (i) CO_2-vasoreactivity is abolished and not reversed by a NO-donor administration, (ii) eNOS and nNOS isoformes expression are significantly decreased ($P < 0.05$), (iii) capillary diameter is significantly increased ($P < 0.05$) (iv) angiogenic and vessels maturation factors (VEGFa, Ang1, TGFβ and PDGFβ) and (v) BBB protein expression mRNA (claudin-5, occludin, ZO-1 and Ve-Cadhérine) are significantly decreased ($P < 0.05$). However, they were not associated with modification of morphology at TEM. After pMCAo at D1 and D7, infarct volume, neurological deficit and neuronal death were significantly increased ($P < 0.05$) in type-1 diabetic mice. At D7, both VEGFa mRNA and endothelial cell proliferation were significantly increased ($P < 0.05$).

Conclusion: Pre-existing diabetes alters cerebral vasoreactivity and NOS isoformes expression; induces vessel destabilization by decreasing angiogenic and vessels maturation factors and BBB protein expression. As a consequence, in diabetic mice, stroke volume and neurological deficit are more severe and angiogenesis repair is delayed.

Keywords: cerebral, ischemia, diabetes, microangiopathy, angiogenesis.

STP, PG, PK, MODÉLISATION

CO-078

Pharmacokinetics and pharmacodynamics of tacrolimus in liver transplant recipients: inside the white blood cells

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Objectives: Some patients experience acute graft rejection while their immunosuppressive drug whole blood concentrations are within the therapeutic range. Tacrolimus (TAC) acts inside the lymphocyte, thus determining TAC intracellular concentrations could be more relevant to predict drug action. Another innovative approach could be to assess the intracellular inhibition of calcineurin, the target protein of TAC. The aim of the present study was to describe the intracellular pharmacokinetics and pharmacodynamics of TAC in liver transplant recipients on day-1 of treatment and at steady-state.

Patients and methods: Eight *de novo* liver transplant recipients treated with TAC were included in the study. They underwent complete pharmacokinetics on day-1 and -7. TAC was measured in whole blood and in cells using liquid chromatography tandem mass spectrometry [1]. Calcineurin activity was measured according to a previously published method [2]. All analyses were expressed for 1 million white blood cells. Results were expressed as means ± standard-deviations.

Results: For whole-blood pharmacokinetics, maximal concentration (Cmax) reached 13.0 ± 9.6 ng/mL at 3 h on day-1 and 8.8 ± 5.0 ng/mL at 1 h on day-7. Corresponding areas under the curve over the administration period (AUC_{0-12}) were 109.9 ± 56.4 ng.h/mL and 81.2 ± 37.2 ng.h/mL, respectively. For intracellular pharmacokinetics, Cmax reached 123.1 ± 151.6 pg/million cells at 3 h on day-1 and 58.9 ± 45.9 pg/million cells at 6 h on day-7. AUC_{0-12} were 855.4 ± 651.7 pg.h/million cells and 519.3 ± 116.5 pg.h/million cells. Trough concentrations were 56.3 ± 30.9 and 24.0 ± 4.1 pg/million cells on day-1 and 7, respectively. Calcineurin inhibition reached a maximum at 3 h (26.5 ± 41.1%) on day-1 but was relatively weak on day-7 (no more than 10%).

Discussion-conclusion: Intracellular TAC concentrations displayed large inter-individual variability. Calcineurin inhibition was weak, consistent with the EC50 reported with whole-blood concentration in the literature (26.4 ng/mL). Further studies should be performed to elucidate the relationship between intracellular concentration of TAC and intracellular calcineurin inhibition.

References:
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2. Blanchet et al, Anal Biochem. 2003; 312(1):1–6.

Keywords: tacrolimus, calcineurin, pharmacokinetics, pharmacodynamics, intracellular, transplantation.

CO-079

Readministration of high dose gentamicin before dialysis in critical care patients

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Objectives: In a previous study[1] we showed that pre-dialysis dosing of gentamicin in ICU patients, *i.e.* administration of the dose just prior to hemodialysis, allowed for high peak concentrations, followed by rapid removal, suggesting better efficiency and less toxicity. No data regarding any subsequent dose have been published in this study or elsewhere, therefore we are reporting here on data from a subset of 6 patients having received two consecutive doses.

Material and methods: Six mg/kg of gentamicin were infused in ICU patients over a period of 30 min(D1). A 4-h hemodialysis session was started 30 min after the end of the infusion. Blood samples were drawn one (C_{max}), 6, 12, 20 h after the start of infusion and every 12 h until concentrations below 2 mg/L were attained. When concentrations dropped below this value, a second 6 mg/kg-dose was administered and the same hemodialysis session and sampling scheme were performed anew(D2). A one-compartment model with zero-order input and first-order elimination was implemented in WinNonlin® to analyze patients' concentrations. The model was parameterized in terms of interdialytic or nonhemodialysis clearance (CL_{NHD}), hemodialysis clearance (CL_{HD}) and volume of distribution (V). Estimated PK parameters were compared between D1 and D2.

Results: Although mean(SD) value of V increased only moderately (0.22 (0.05) vs. 0.26 (0.06) L/kg) between D1 and D2, marked intra-individual variations were observed. V could decrease or more frequently increase as much as 50% but variations did not impact C_{max}/MIC ratios which remained above 10. While CL_{HD} remained constant (82.9 (22.8) vs. 89.4 (15.2) mL/min), CL_{NHD} varied widely but remained very low in all cases (≤20 mL/min). C_{24} results were similar (4.4 (1.5) vs. 4.4 (1.3)) but readministration was not allowed at the time and had to be delayed [36–80 h post-dosing].

Discussion: Data regarding readministration of gentamicin in this situation are scarce. We have shown in this study that readministration of a second high-dose gentamicin before dialysis is possible. Due to marked intra-individual variability and to highly variable readministration time, we can confirm that therapeutic drug monitoring is mandatory in these patients.

Reference:

1. Antimicrob Agents Chemother, 2013, 57(2):977–82.

Keywords: aminoglycosides, hemodialysis, pharmacokinetics, therapeutic drug monitoring.

CO-080

Optimization of sorafenib dosing regimen using a utility function based on efficacy, toxicity and system's pharmacology.

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Objectives: The utility function allows finding a compromise between drug efficacy and toxicity, balancing the probability of benefit and the probability of risks [1,2]. Sorafenib is an oral non-specific multi-kinase inhibitor, approved for the treatment of renal and hepatic carcinoma, blocking cell proliferation and angiogenesis by targeting Raf/ERK pathway. Hand-foot Syndrome (HFS) is one of the major dose-limiting toxicity. This work aimed at applying the concept of utility function to determine the optimal regimen of sorafenib, integrating multi-scale models for efficacy and toxicity.

Methods: Several data and models from the literature were linked, considering different scales of space (intracellular pathways to populations of patients) and different scales of times (minutes–months): (i) the non-linear plasma pharmacokinetics of sorafenib[3]; (ii) the sorafenib intracellular uptake[4]; (iii) the Raf/ERK signaling pathway[5]; (iv) the inhibitory potency of sorafenib for Raf[6]; (v) the sorafenib induced HFS[7]. The criterion for efficacy, to be maximized, was defined as the sustained decrease in the area of the peak of bi-phosphorylated ERK (ERKPP). The criterion for toxicity, to be minimized, was defined as the risk for severe HFS. The optimal regimen was defined as the one maximizing the utility score.

Results: Simulations of various dosing regimen allowed computing the surface of the utility function under different regimen (daily amount vs. frequency). The usual sorafenib regimen 400 mg bid was found among the most utile. The more the daily dose is fractionated, the more efficacy and toxicity risks increase; for regimens above 800 mg split on 4 occasions, the increased risk for severe toxicity is not compensated anymore by a gain in efficacy, and utility decreases.

Conclusion: The utility function through the integration of multi-scale models allowed the optimization of the administration of targeted anticancer agents such as sorafenib. This approach will be extended, in future steps, to include several toxicity risks and to individualize the utility score according to patients' preferences on efficacy and toxicity[8].

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Keywords: utility score, multiscale, oncology, efficacy, toxicity.

CO-081

Evaluation of the effect of antiviral on the transport of tenofovir by hOAT1 and hOAT3

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Objectives: Human immunodeficiency virus 1 (HIV-1) infections are treated with combined administration of antiretroviral drugs. Those drugs improve patient outcomes while increasing the likelihood of drug interactions. The active renal secretion of tenofovir (TFV) a HIV nucleotide reverse transcriptase inhibitors across proximal tubules occurs via uptake by human organic anion transporters, OAT1 (SLC22A6) and OAT3 (SLC22A8). They are also the principal OATs in the renal proximal tubule. Co-administration of some HIV inhibitors with tenofovir disoproxil fumarate, an oral prodrug of TFV, has been shown to increase systemic levels of TFV. The purpose of this study was to evaluate the inhibitory effect of several antiviral drugs in the transport of TFV by OATs.

Methods: OAT1 and OAT3 inhibition were assessed by *in vitro* models (HEK Cells). We tested the level of [^{3}H]TFV accumulation in HEK hOAT1 cells and KEH hOAT3 respectively and compared with corresponding control cell line HEK Mock.

Results: We assessed the uptake of [^{3}H]TFV on hOAT1 and hOAT3 HEK cells. We obtained a higher affinity for OAT1 than OAT3. Thus we tested the inhibitory effect of the following antivirals drug on hOAT1HEK: Tipranavir, zidovudine, atazanavir, nevirapine, lopinavir, ritonavir, lamivudine and ribavirin at concentrations ranging from 0 to 100 μM. Only three of them showed a statistically significant inhibition of [^{3}H]TFV on hOAT1 HEK cells: IC_{50} Tipranavir ≪ Zidovudine IC_{50} 50. Interestingly, those IC_{50} were in the range of their therapeutic levels.

Conclusions: These data indicate a low potential of antivirals drug to interfere with the active tubular secretion of TFV and to alter the clinical renal safety profile of TDF.

Keywords: tenofovir, hOAT1, hOAT3, drugs interactions.

CO-082

Individualization of high dose carboplatin with therapeutic drug monitoring and pharmacogenetic markers of toxicity in TICE protocol (testicular germ cell lines)

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Objectives: The TICE protocol is a national phase II multicentre trial aiming to evaluate the efficacy (primary endpoint) and tolerance of Paclitaxel plus Ifosfamide followed by individualized high-dose carboplatin plus etoposide treatment in previously treated germ cell tumors. The particularity of the trial (in comparison with the standard protocol proposed by Motzer [1]) is that the carboplatin dose is individualized for each patient according to therapeutic drug monitoring (TDM) in order to reach the target AUC of 24 mg/min/mL over 3 days.

Patients and methods: Sixty one patients were included between March 2009 and April 2012, when the study was stopped for planed intermediate analysis. In the intensification phase, patients were treated for 3 consecutive days with carboplatine/etoposide during 3 cycles. Pharmacokinetic samples were taken at day 1 in order to estimate the carboplatine clearance by a Bayesian approach using NONMEM and adjust the dose at day 3 to reach the target AUC of 24 mg/min/mL over 3 days. Moreover, we conducted a pharmacogenetic study to identify genetic polymorphisms associated with toxicity.

Results: We retrospectively analyzed the total AUC and assessed the intra- and inter-cycle variability observed for carboplatine exposure. We observed that the equation previously developed and validated in the general population treated with carboplatin [2] overestimated carboplatine clearance in this particular patient group (young male adults). We assessed the performances of different methods of carboplatine dose individualization (in the perspective to widespread this protocol) and showed that TDM remained the most accurate method. Pharmacogenetic analyses showed that one SNP was associated with the development of ototoxicity at the first cycle of carboplatin.

Discussion: This work shows the benefit of using TDM to individualize carboplatin dose to reach the recommended target AUC of this high dose chemotherapy regimen. The clinical trial is ongoing for inclusion of 36 additional patients for final evaluation.

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Keywords: therapeutic drug monitoring, high dose carboplatin, pharmacogenetics.

CO-083

Lean body mass and early therapeutic drug monitoring may help to optimize sunitinib dose in cancer patients

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Background and objectives: Sunitinib is an oral targeted therapy with a high interindividual pharmacokinetic variability. The contribution of the lean body mass (LBM) and pharmacogenetic variants to sunitinib exposure has not been yet investigated. Besides, the association between sunitinib exposure and clinical outcomes has not been yet fully elucidated. The aims of this retrospective study were to assess the influence of the LBM and pharmacogenetic variants on the exposure to sunitinib and SU12662 (active metabolite), and the relationship between clinical outcomes (severe toxicities, survival), drug exposure and pharmacogenetic variants in 92 adult outpatients with solid tumours treated with single-agent sunitinib.

Methods: Sunitinib and SU12662 exposure was assessed during the first treatment cycle (days 10 and 21). Meanwhile acute toxicity was graded using the NCI 4.0 CTCAE ver. 4.0. Clinical characteristics and 14 common single nucleotide polymorphisms in the *CYP3A4/3A5, NR1I2, NR1I3, ABCB1,* and *ABCG2* genes were analyzed according to the exposure to sunitinib and SU12662. Associations between clinical characteristics, drug exposure and pharmacogenetic variants with toxicities were assessed using a multivariate analysis. The relationship between drug exposure and survival was assessed in 55 renal cell carcinoma (RCC) patients using the Kaplan–Meier method.

Results: Two major independent variability factors of composite (sunitinib + SU12662) exposure were identified: LBM ($P < 0.0001$) and *ABCG2* (421C>A) ($P = 0.014$). Together, they explained 32.4% of the interindividual variability, with LBM accounting for 23.5%. Older age (OR = 1.47 [1.01–2.15], $P = 0.048$) and high sunitinib exposure (OR = 1.16 [1.05–1.28], $P = 0.005$) were independently associated with any grade ≥ 3 toxicity, and high SU12662 exposure with grade ≥ 2 thrombocytopenia (OR = 1.27 [1.03–1.57], $P = 0.028$). The overall survival was longer in RCC patients with high composite exposure (AUC; >1.973 ng/mL/h) at day 21 than in patients with low exposure (1057 vs. 501 days, respectively; log-rank $P = 0.0051$).

Conclusions: The present study highlights the major contribution of the LBM in the high interindividual pharmacokinetic variability of sunitinib. It shows that severe toxicities (any grade ≥ 3 toxicity, grade ≥ 2 thrombocytopenia), as well as the overall survival in RCC patients were related to the drug exposure. Overall, these results suggest the clinical usefulness for individualized dose regimens based on a drug monitoring to optimize sunitinib use.

Keywords: sunitinib, toxicity, efficacy, drug exposure, lean body mass, pharmacogenetics.

CO-084

R/S Ibuprofen levels in preterm infants with hemodynamically significant ductus arteriosus: data in real life

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Hemodynamically significant ductus arteriosus (hsPDA) is a frequent complication in preterm infants. A racemic melange of ibuprofen (IBU) formulated with trometamol (PEDEA®) is used in France for pharmacological closure of hsPDA. We present here a prospective real life study in preterm infants in which iterative determination of the two enantiomers were performed.

Methods: The study population consisted of 54 preterm infants.

Results: The mortality rate was 30%, mainly due to intra-ventricular haemorrhage (HmIV) and/or sepsis. The closure rate was 50%. Only 17 children received at least 3 doses. The mean cause of premature discontinuation of treatment (18/21) was side effects (SE) (5 digestive, 14 renal, 14 HmIV). Mean S-IBU level was 13.2 ± 3.0 mg/l with only traces of R-IBU if present. We retrieved a significant positive correlation between S-form levels and weight birth, a negative one between S-form levels and precocity of ibuprofen treatment and albumin level. No correlation was found when we compared ibuprofen levels and closure of hsPDA. As expected, amikacin exposure doubled the occurrence of an increase in creatininemia ($P < 0.009$).

Discussion: Despite levels comparable to a Cmax at around 30 mg/l with estimated half-life of 30 h, we confirm the absence of correlation between IBU concentrations and hsPDA closure. Therefore, we observed in real life a lesser efficacy of IV ibuprofen treatment than those expected in clinical trials (about 70–80% of closure). Long-term studies are needed to better evaluate the benefit/risk ratio of IBU in preterm infants, whatever the mode of administration.

Keywords: ibuprofene, ductus arteriosus, concentration, outcome.

CO-085

Understanding the dose-response relationship of a pro-apoptotic compound in mice through PK-PD modeling

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Background: The increasing knowledge on cellular biology and pathophysiological processes allowed the characterization of receptors and signaling cascades whose deregulation may lead to tumorigenesis, such as the loss of apoptotic capability, through the inhibition of Caspase. A compound has been evaluated as a pharmacological tool in non-clinical settings, to restore apoptosis functions. Based on data issued from *in vitro* experiments and *in vivo* studies in mice, the aim of this work was to characterize PK-PD relationships between dose, plasma concentration and Caspase activity.

Methods: Data from preclinical PK and biomarker studies at 8 dose levels on xenografted mice were considered for population modeling approach. 218 mice were used for PK studies and 54 for biomarker analysis. The pro-apoptotic effect was defined by an enhanced activation of Caspase. This was resulting from the reversion of Caspase inhibition, through target-receptor occupancy. The effective concentration for target-binding was linked to plasma concentration, considering the unbound fraction and target affinity, derived from *in vitro* experiments. Model development was guided by residual- and simulation-based diagnostics. From this model, simulations at different doses were performed to probe Caspase mechanism.

Results: PK and Caspase kinetics were successfully characterized by the proposed PK-PD model. The pharmacokinetics was described by a two-compartment disposition model with both saturable absorption and elimination. Auto-induction observed with repeated administrations in mice was characterized by the stimulation of the production of the (hypothetic) enzyme involved in elimination processes. Caspase was found to be activated from a threshold effective concentration, maximal activation being rapidly reached (bistable on/off activation). Increasing the administered amount does not allow a higher but a more sustained activation level.

Conclusion: The proposed PK-PD model for plasma concentration, target-binding and Caspase activity will be extended to be connected to tumor growth dynamics in xenografted mice. Such model would provide a powerful tool to quantify the expected antitumor effects and to propose an optimal dosing regimen in mice.

Keywords: pro-apoptotic compound, PK-PD modelling, biomarker, xenografted mice.

CO-086

Population pharmacokinetics of colistin in critically ill patients: the loading dose rationale revisited

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Background: Colistin, often the last-resort treatment for multidrug-resistant gram-negative bacteria, is administered as the inactive prodrug colistin methanesulfonate (CMS). As CMS is largely excreted unchanged in urine, concentrations of colistin increase when the renal function deteriorates. We aimed to characterize the renal clearance of CMS in critically ill patients from both plasma and for the first time from urine samples and to confirm previous pharmacokinetic results already published in this population of patients.

Methods: Seventy-three (73) critically ill patients were included and treated with a CMS dosage regimen chosen by the physician, a total of 634 plasma and 38 urine samples were collected.

Results: We confirmed that renal clearance of CMS was related to creatinine clearance. The typical C_{max} of colistin after the first median dose of 2 MIU was about 2 mg/L, $t_{1/2}$ of colistin was 3.1 h and steady-state was reached within few hours.

Conclusions: Our results are partially consistent with those recently published. Due to the excretion of CMS measured in urine, maintenance doses should indeed be adapted to renal function. However, following the initial CMS dose, the observed concentrations of colistin were several folds higher than those previously reported and steady-state was reached much more rapidly, possibly due to the various CMS brands. These discrepancies challenge the pharmacokinetic rationale for a loading dose.

Keywords: colistin, pharmacokinetics, loading dose, critically ill patients.

CO-087

Azole antifungals: therapeutic drug monitoring and/or bioassay?

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Background: As the incidence of invasive fungal infection is constantly growing, failure of antifungal treatment remains a concern. Azole antifungals present high intra and interindividual variabilities of their plasmatic concentrations (C_{min}), justifying their therapeutic drug monitoring (TDM). Nevertheless, neither the minimal inhibitory concentration, nor the C_{min}, can certify the effective antifungal activity (AA) expected for each patient. The aim of this study was to develop a technique allowing the determination of AA, to assess the influence of plasma on AA and to correlate AA to C_{min} in plasma patients.

Patients/Methods: The bioassay consists of the determination of AA on *Candida parapsilosis* using disk diffusion method. Standard curves were determined for posaconazole (PZC) and voriconazole (VRC) in water and 45% of plasma to assess the influence of plasma on AA. Plasma of patients currently undergoing TDM for PZC ($n = 74$) or VRC ($n = 78$) were prospectively collected for AA determination. The best fit of the C_{min} plotted against AA was obtained with a cubic regression for both antifungals, for standard and plasma patient curves according to the equation: $AA = B_0 + A_1[C_{min}] + A_2[C_{min}]^2 + A_3[C_{min}]^3$ with B_0 = intercept.

Results: In water, plasma and patient samples, cubic regression between AA and C_{min} indicated R^2 values of 0.997, 0.999 and 0.819 for PZC and of 0.996, 0.990 and 0.925 for VRC ($P < 0.001$ for each curve). Standard curves of VRC or PZC without or with plasma adjunction did not differ (Wilcoxon-test: $P = 0.79$ for VRC, $P = 0.87$ for PZC), suggesting that plasma protein has no effect on antifungal activity in the proposed disk method. For VRC, C_{min} of 1 and 4.7 mg/L corresponded to 54% and 90% of maximal AA respectively. For PZC, C_{min} of 0.5, 0.7 and 1 mg/L respectively corresponded to 26%, 40% and 53% of maximal AA.

Discussion: The proposed disk model was validated considering the relationships between AA and C_{min}. AA determined with the bioassay could be useful to better characterize therapeutic range, particularly the lower one for PZC and the upper one for VRC. The bioassay brings additional information to complete TDM interpretation, especially in patients with low C_{min} values.

Keywords: azole antifungals, therapeutic drug monitoring, bioassay, voriconazole, posaconazole.

CO-088
High imatinib dose overcomes insufficient response associated with ABCG2 haplotype in chronic myelogenous leukemia patients

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Pharmacogenetic studies in chronic myelogenous leukemia (CML) typically use a candidate gene approach. In an alternative strategy, we analyzed the impact of single nucleotide polymorphisms (SNPs) in drug transporter genes on the molecular response to imatinib, using a DNA chip containing 857 SNPs covering 94 drug transporter genes. Two cohorts of CML patients treated with imatinib were evaluated: an exploratory cohort including 105 patients given 400 mg/d and a validation cohort including patients sampled from the 400 mg/d and 600 mg/d arms of the prospective SPIRIT trial ($n = 239$). Twelve SNPs discriminating patients according to cumulative incidence of major molecular response (CI-MMR) were identified within the exploratory cohort. Three of them, all located within the ABCG2 gene, were validated in patients included in the 400 mg/d arm of the SPIRIT trial. We identified an ABCG2 haplotype (defined as G-G, rs12505410 and rs2725252) associated with significantly higher CI-MMR in patients treated with 400 mg/d. Interestingly, we found that patients carrying this ABCG2 'favorable' haplotype in the 400 mg arm reached CI-MMR rates similar to those of patients randomized in the imatinib 600 mg/d arm. Our results suggest that response to imatinib may be influenced by constitutive haplotypes in drug transporter genes. Lower response rates associated with 'non-favorable' ABCG2 haplotypes may be overcome by increasing the imatinib daily dose up to 600 mg/d.

Keywords: pharmacogenetic, CML, ABCG2, SNPs, imatinib, CML major molecular response.

CO-089
A Bayesian approach to assess glomerular filtration rate (GFR) using plasma iohexol clearance in renal transplant recipients

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Objective: Accurate glomerular filtration rate (GFR) evaluation is crucial in kidney transplant recipients. The plasma clearance of iohexol ($CL_{iohexol}$) is a good surrogate for GFR. However, in routine practice, $CL_{iohexol}$ is estimated using 4–6 blood samples collected in the elimination phase, and then corrected using the Bröchner-Mortensen (BM) formula to overcome the lack of information on the distribution phase. However, this formula has never been validated in this specific population.The aim of this study was to develop and validate a bayesian model using a limited sampling strategy for estimating GFR in kidney transplant recipients and to study whether it can be used instead of the BM formula.

Patients and methods: Six blood samples were drawn from each patient according to 3 different samples schemes, allowing description of the bi-exponential decay of iohexol concentrations. Patients were then separated into an index set and a validation set. A two-compartmental PK-POP model was developed in the index set to estimate $CL_{iohexol}$. Using this PK-POP model we determined the individual (posthoc) $CL_{iohexol}$, which approximates the individual reference GFR (GFR_{ref}). Then, several limited sampling strategies (with 2, 3 or 4 samples per patient) were tested in the validation set. The Bayesian estimates of $CL_{iohexol}$ were compared to GFR_{ref} by analysis of bias (me) and precision (rmse). In parallel, GFR estimated using the BM formula was compared with GFR_{ref} in the entire population.

Results: From the 155 patients included, 104 were allocated to the index set and 51 to the validation set. Covariate analysis showed that uremia was significantly associated with $CL_{iohexol}$ ($P < 0.001$) and BSA (body surface area) with V1 ($P < 0.001$). The strategy with samples drawn at times 5, 60 and 270 min allowed accurate estimation of $CL_{iohexol}$ (i.e. GFR) (mean bias: −2.2%, mean imprecision: 8.5%). The BM equation estimated GFR with mean bias of −3.0% and mean imprecision of 7.2%.

Discussion: This Bayesian model allows the determination of GFR with a similar accuracy as the BM equation. Thus, this strategy based on a lower number of samples should improve patient and caregivers comfort.

Keywords: glomerular filtration rate, iohexol, Bayesian estimation, population pharmacokinetics.

COMMUNICATIONS EN PHARMACOLOGIE

CO-090
Why are efflux inhibitor – cytotoxic drug combinations effective in animal studies but not in clinical trials ?

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Background: Studies with efflux inhibitor – cytotoxic drug combinations against cancer usually show good efficacy in animal studies but, at best, moderate efficacy in clinical trials. The reasons for this discrepancy were assessed by modelling and simulation.

Methods: A pharmacokinetic-pharmacodynamic model of tumor growth inhibition by this drug combination was set up. The model was evaluated by simulation and reanalysis of an animal study of the doxorubicin-fluoxetine combination [1] and a clinical trial of daunorubicin-zosuquidar in acute myeloid leukemia [2].

Results: The conditions that maximize treatment efficacy are a low activity of the efflux transporter, and a high and prolonged inhibitor exposure. The major condition to maximize the difference between the placebo and the efflux inhibitor arms is a high transporter activity. In the study on fluoxetine, animals were xenografted with tumors expressing PgP at a very high level (ratio of doxorubicin extracellular to intracellular concentration >30) so that fluoxetine had a major impact on doxorubicin efficacy. In the daunorubicin – zosuquidar clinical trial, the proportion of patients with high PgP activity (ratio of daunorubicin extracellular to intracellular concentration >4) should have been 40% or higher to observe a clear difference in complete remission rates between the placebo and zosuquidar arms. But in the clinical trial, the median concentration ratio was 2.5. Simulations showed that the activity of PgP was too low in most patients of the trial to allow detection of a difference between the placebo and efflux inhibitor arms.

Conclusions: Maximizing treatment efficacy or demonstrating the effectiveness of an efflux inhibitor require opposite tumor properties. Animal studies gave an optimistic view of the impact of PgP inhibitors, because PgP activity was much higher than the level encountered in most patients. But PgP inhibitors could be effective for patients bearing tumors with a high efflux rate.

Reference:
1. Peer D et al. Cancer Res. 2004;64(20):7562–9.
2. Cripe LD et al. Blood. 2010;116(20):4077–85.

Keywords: P-glycoprotein, efflux inhibitors, resistance, tumor growth model, trial simulation.

CO-091
Simulation of an interaction between ciprofloxacin and probenecid using the mechanistic kidney model of SimCYP®

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Objectives: To test the mechanistic kidney model recently added in SimCYP® version 12 using simulation of a drug-drug interaction (DDI) study between ciprofloxacin and probenecid.

Material and methods: The software used is SimCYP® version 12. The values of ciprofloxacin's physicochemical properties implemented in the physiologically based pharmacokinetic (PB-PK) model was found in the literature [3,4]. The reference data is a DDI clinical trial published in 2010 [1], 12 healthy volunteers, 50% women, aged between 21 and 38 years old inclusive, randomized cross-over study with 2 arms and 2 periods. The trial design in SimCYP® was the same as in the publication except that the simulation was made on 10 trials of 10 subjects. The validation of ciprofloxacin (OAT3 substrate) model was done on simulated PK profiles and PK parameters, and the validation of probenecid (OAT1-OAT3 inhibitor) model on concentrations profiles, compared to reference data.

Results: The final ciprofloxacin model well described published plasma and urine data after single administration, and PK parameters were correctly estimated. The probenecid model well described published plasma data after single [2] and repeated administration. Observed data in plasma and urine in the interaction study were adequately described by the model, the simulated PK parameters were close to the observed parameters, and the average AUC and C_{max} inhibitory ratios were in the same order of magnitude. The ratios of the mean amount of ciprofloxacin excreted in urine over 24 h were also very similar between the simulations and the published data.

Discussion-Conclusion: Probenecid is an inhibitor of OAT1 and OAT3 as well as CYP2C9 and 2C19, and inducer of 2C8 and 3A4 but only OATs data were implemented in the model. For ciprofloxacin model any impact of hepatic metabolism was taken into account, and a global OAT/Km estimation was implemented on OAT3. As for the DDI between ciprofloxacin and probenecid, SimCyp® kidney model can be used for clinical DDI risk assessment for compounds under development involving OATs, either as substrates or potential inhibitors.

References:
1. Landersdorfer CB *et al.* Br J Clin Pharmacol 2010;69:167–78.
2. Emanuelsson BM *et al.* Eur J Clin Pharmacol 1987;32:395–401.
3. Olivera ME *et al.* J Pharm Sci 2011;100:22–33.
4. Dictionnaire Vidal 2012.

Keywords: physiologically based pharmacokinetic, SimCYP version 12, mechanistic kidney model, drug-drug interaction, organic anion transporters.

CO-092
Guideline for optimization of the pharmacovigilance of the radiopharmaceutical in clinical trials

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Introduction: Radiopharmaceuticals (RP) are drugs containing radionuclide and carrier molecules which bind to determinate targets. The large opportunities offered by their use in both diagnosis and therapeutic, lead to the development of many clinical trials. A guideline was needed to help the pharmacovigilance responsible person and the study manager to have a rational and complete approach of the risk and respect the regulation.

Method: The sources of information were:

1. The laws and rules established by the authorities for drug regulation as well as these for radionuclide regulation, and for radioprotection regulation.

2. The published data upon pharmacological properties and adverse effects of the main isotopes and of the most frequently used carrier molecules.

Results: The document gives a list of points to consider and provides advice for:

1. Characterization of the RP: type of isotope, nature of the carrier agent, information upon procedure of the production and distribution,

2. Description of the use of the RP in the study: use for diagnosis use for diagnosis or therapy, activity injected, route and frequency of administration, total duration of exposition, calculation of patient dosimetry, type of imaging,

3. Identification of the target population, special situations as children, elderly, or critical ill subjects,

4. Identification of the exposure of the family or relative as well as professionals,

5. Adaptation of the list of the expected adverse drug reactions and description of the supervision procedures,
6. Mandatory reporting rules.
Conclusion: The pharmacological profile of these drugs and the regulatory complexity due to their dual membership require a rigorous supervision of these trials. This guideline, which proposes a frame to conduct thinking and ensure consideration of the integrity of regulatory issues and data safety, provides support easy to use.
Keywords: radiopharmaceuticals, pharmacovigilance, clinical trial, guideline.

CO-093

Trends in methylphenidate dispensing from 2005 to 2011

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Objective: Methylphenidate is approved for children aged six and over in the treatment of attention deficit hyperactivity disorder and for adults in the treatment of a rare disease, narcolepsy. Available in France since 1996, its prescription is under restrictive conditions because of its abuse potential described and reported in the literature. Besides, methylphenidate sales have been increasing extensively since 2004. In this context, the aim of this study is to describe patterns of methylphenidate use.
Method: Data for this study were provided by the drug reimbursement database of the General Health Insurance System (3 860 000 persons in 2011). All individuals from Provence-Alpes-Côte d'Azur-Corse having at least one dispensing of methylphenidate between 2005 and 2011 were selected. In addition, psychoactive medications dispensed during methylphenidate dispensing timeframe were analyzed.
Results: The number of individuals having at least one dispensing per year of methylphenidate has shown a 166.1% growth from 2005 to 2011. During this period, the proportion of male decreased from 80.0% in 2005 to 73.3% in 2011 ($P < 0.001$). Mean age increased from 15.4 ± 12.5 in 2005 to 17.5 ± 13.5 years old in 2011 ($P < 0.001$). The proportion of adults raised significantly, from 14.8% in 2005 to 24.7% in 2011. Among adult population, in 2011, 55.1% of the dispensings were for male patients, and there mean age was 37.9 ± 12.7 years old. Concerning psychoactive medication dispensings, 53.3% of them had benzodiazepines, 33.6% antidepressants, 28.1% opiate maintenance treatment, 27.0% antipsychotics, 11.8% morphine, and 2.6% modafinil.
Discussion and conclusion: Methylphenidate dispensing increase is confirmed in this study. Moreover, the difference between the target population and the population joint was underlined, particularly considering adults. These data may be considered as a significant 'signal' that should be investigated by drug utilization and safety studies.
Keywords: methylphenidate, usability study, pharmacoepidemiology.

CO-094

Benzodiazepines new use and long-term cognitive decline in elderly: a prospective cohort study

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Introduction: Several studies have associated benzodiazepine use to an increased risk of dementia in the elderly. The mechanism underlying this association, which would relate to a major public health impact if causal, remains unknown. Some studies showed an accelerated cognitive decline in benzodiazepine users while others did not. However, none considered in their analyses the putative association between benzodiazepine use and dementia, despite the cognitive decline related to dementia could jeopardize the estimation of the intrinsic role of benzodiazepine on cognition.
Objective: To evaluate the effect of new benzodiazepine use on long-term cognitive decline in initially non-demented elderly, considering the concomitant risk of dementia.
Methods: A study was conducted in a cohort of elderly identified among the participants of the prospective PAQUID study. Among the non users of BZD at baseline and without dementia at follow-up 3 years (T_3) in PAQUID, 167 BZD incident users and 1136 non users were identified. Temporal trend of their cognitive function was investigated by considering results obtained at the Mini Mental State Examination (MMSE) and Isaacs Set Test (IST) at follow-up visits conducted every 2–3 years after T_3. The association between incident BZD exposure and the evolution of cognitive performance was studied using non-linear mixed models considering a latent process as dependent variable. These models were adjusted for constant variables (age, sex, educational level), and time-dependant ones (wine consumption, celibacy, dementia, use of other psychotropic, and use of cardiovascular drugs).
Results: Adjusted models found no longitudinal effect of benzodiazepine new use on cognitive decline neither evaluated using MMSE (estimate = 0.02 and $P = 0.26$), nor using IST (estimate = 0.03 and $P = 0.10$). The cognition level over follow-up and the cognition evolution trend appeared similar between benzodiazepine new-users and non-users.
Conclusions: The incident BZD exposure was not associated with a difference in the temporal trend of cognitive performance, neither on short-term or on long-term. Our study considering the concomitant risk of dementia did not objective a proper effect of benzodiazepine new use on cognitive level or decline in the elderly.
Keywords: alzheimer's disease, dementia, cognition, benzodiazepines, elderly.

CO-095

Association between benzodiazepine drugs and total mortality: evidence from a study in the EGB

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Objectives: Benzodiazepines and related drugs could be involved directly in fatal outcomes, such as in mixed drug overdose, car crashes, or suicide attempts, even if the ways by which these drugs can lead to an increased mortality are not entirely elucidated. This study intended to investigate the impact of benzodiazepines exposure on all-cause mortality.
Material and methods: A retrospective exposed unexposed cohort study was conducted in the French reimbursement database EGB from 2006 to 2012. Subject aged 18 years or older, and registered in the database for at least 1 year, were eligible. Exposed patients were new users of benzodiazepine, with no benzodiazepine nor antidepressants or non-benzodiazepine anxiolytics/hypnotics in the past year. Exposed patients were matched to one control in each of the 2 control groups (new users of antidepressants in cohort 1, new GP consultants with no GP consult in the past year in cohort 2) according to birth year, and gender. Time to death was analyzed using an extended Cox regression model with time-dependent covariates.
Results: The population comprised 171 861 patients (57 287 by group). Female were 63%, mean (SD) age was 47.5 (18.3). New users of alprazolam, bromazepam, zolpidem and zopiclone were mainly represented. After adjustment on clinical covariates, 12 months all-cause mortality was found to be significantly higher among those exposed to benzodiazepines, [HR: 1.26, 95% CI 1.08–1.48, $P = 0.004$], but not significantly higher in control 1 [HR: 1.07, 95% CI 0.91–1.27, $P = 0.402$], compared to controls 2 as the reference. When considering benzodiazepines as a time-dependent covariate, HR was 1.24 [95% CI 0.99–1.54, $P = 0.061$].
Conclusions: This study enables to bring additional evidence on the relation between benzodiazepine use and all-cause mortality. The apparent high overall increase in risk is largely attenuated after adjustment for a large set of confounders, and benzodiazepines do not appear to lead to more than a mild increase in all-cause mortality. Moreover these results are consistent with those from a study performed simultaneously in the UK CPRD, even if the risk is lower in the French EGB.
Keywords: benzodiazepine, mortality, cohort, database.

CO-096

Inappropriate prescription of benzodiazepines in patients with alcohol-induced disorders

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Introduction: Benzodiazepines (BZD) are currently used to prevent incidence and severity of alcohol withdrawal syndrome. According to French recommendations (HAS 1999) BZD treatment duration should not exceed 10 days. The aim of this work is to describe the details of benzodiazepines deliveries in a cohort of alcohol-dependent patients.
Method: This retrospective cohort study used the Sample Generalist Beneficiaries (EGB), a representative sample 1/97th beneficiaries of the French national health-care insurance database, currently composed of more than 600 000 subjects. Patients included were men and women ≥15 years who received a first delivery (index date), without refund within previous 12 months, one of the three treatments approved to maintain abstinence in alcohol-dependent patients: acamprosate, naltrexone and disulfiram, between 01/01/2007 and 31/12/2011. Consumption of BZD patients were followed until 31/12/2012. Patients who received BZD before the index date were excluded. Interruption of treatment ≥90 days defines the end of a cure.
Results: 1223 patients showing a first treatment were included. Among them, 732 patients underwent an associated prescription of BZD (60%), predominantly male (80%), mean age 44 ± 11 years [16–78]. The main BZD issued were oxazepam (36%), alprazolam (21%) and diazepam (19%). Anti-craving drugs were mainly prescribed by generalist physicians (98%) and basically acamprosate (71%). 83% received BZD and anti-craving drug delivery the same day ($n = 611$). In 17% of cases, this delivery is delayed with a median of 36 days of initiation [18–72]. The delivery of BZD is only in 51% of patients ($n = 370$). The median duration of the first cure of BZD is 78 days [35–204] and 90 days for the anti-craving drugs [44–213].
Conclusion: Concomitant use of an anti-craving drug with a BZD in the maintenance of alcohol abstinence seems to be a common practice with a higher duration of use compared to recommendation (78 vs. 10 days) with oxazepam in first line therapy (diazepam recommended). The risk-benefit ratio of this association remains to be assessed, taking into account the risk of BZD dependence and the place of these drugs in the new strategies for treatment of alcohol dependence such as controlled drinking.
Keywords: alcohol-induced disorders, benzodiazepines, prescription drug misuse, alcohol withdrawal.

CO-097

Adverse effects and misuse of nefopam: an analysis of French Pharmacovigilance database

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Objective: Nefopam is a non-opioid analgesic approved in France for acute pain, especially postoperative pain. Previous studies have shown that oral misuse of parenteral form is frequent and that adverse drug reactions (ADRs) of nefopam administered per os or injected are broadly consistent with Summary of Product Characteristics (SmPC). The aim of this follow-up was to complete the review of ADRs from 2007, to compare ADRs between administration routes and to assess oral misuse.

Methods: All ADRs notified with nefopam to the French Pharmacovigilance Centres between January, 2007 and December, 2011 were analyzed.
Results: 433 cases of ADRs had been reported. Administration routes were parenteral (73%), oral (15%) or unknown (12%). Safety profile of these cases was consistent with SmPC. Side effects were mostly psychiatric (hallucinations), gastro-intestinal (nausea, vomiting), neurological (drowsiness, dizziness), renal (urinary retention), cardiac (tachycardia) and hypersensitivity reactions (urticaria, anaphylactic shock, angioedema). Safety profiles were similar between oral and parenteral use. However, it seemed that immunoallergic, neuropsychic and cutaneous effects were more frequent with parenteral use than with oral use. Digestive effects seemed more frequent with oral use. Among 98 cases where nefopam was the only suspected drug, unexpected ADRs had been reported, mostly cutaneous eruption with or without pruritus, atrial fibrillation, and unexpected neuropsychic side effects (confusion, delirium, disorientation…). There was no fatal case where nefopam was the only suspected drug. In one fatal case, nefopam was the most suspected drug. The patient experienced urinary retention and acute renal failure which led to fatal morphinic overdose.
Discussion: Safety profile of both oral and injected nefopam was consistent with SmPC. Parenteral use of nefopam seemed to be associated with more immunoallergic, neuropsychic and cutaneous ADRs and oral use with digestive ADRs. Unexpected ADRs had been reported, but did not constitute a safety alert. Oral misuse of nefopam was frequent. According to these results, commercialization of an oral form in France could be interesting, knowing that this form is already available in some other countries and that therapeutic arsenal in pain relief is reduced.
Keywords: nefopam, misuse, French pharmacovigilance database.

CO-098

Psychoactive substances use in a working population in Lorraine: what and why?

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Objectives: Over the last years, there is concern in France about the use of licit and/or illicit psychoactive substances in the workplace. In this context, we proposed a survey in a sample of employees in Meurthe-et-Moselle, Lorraine region, France. What and why do they actually consume? Does consumption vary according to age, occupational status, or with the existence of a pre-existing pathology?
Method: A prospective descriptive survey was conducted by the Center for Evaluation and Information on Pharmacodependence-Addictovigilance (CEIP-A) at Nancy University Medical Center. Data were collected through an anonymous questionnaire submitted to 400 employees in the waiting room before their occupational medical examination. In addition to sociodemographic and professional characteristics, all participants were asked about the use of any drug and reasons for its consumptions.
Results: Among the 347 participants, 56.7% reported consuming products: alcohol (68.0%), cannabis (12.9%), anxiolytics (9.0%), antidepressants (5.9%), cocaine (2.0%), Subutex® (1.6%) and other unspecified substances (0.8%). Older employees consumed more frequently tranquilizers and antidepressants while the younger more frequently consumed cannabis. Among the reasons cited for these consumptions, enjoyment came first (65.0%), followed by stress (18.8%) and overwork (8.5%). 'Being more productive' was reported by only 2.2% of respondents. For 'feel fun' was more frequently reported by those aged 20–29, while 'to combat stress' was more frequently reported by 30–39 years old. Finally, products consumption habits varied according professional sectors: cannabis was mostly used in industry, alcohol in transport, cocaine in trade and tranquilizers in the administration.
Discussion: Our results were generally in agreement with those of similar French surveys [1,2]. A national survey would be necessary in order to confirm these interesting results. This study is of great importance in order to inform clinicians about the use of various psychoactive substances in workplace. In addition, a sensitization campaign for workers about the health risks of these consumptions is highly recommended and could be conducted by occupational physicians.
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2. O.Boeuf-Cazou *et al.* Thérapie, 2011, 66 (2): 155–165.
Keywords: psychoactive substances, workplace, consumption habits.

CO-099

Consumption of tramadol in outpatients: use and misuse

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Objective: Tramadol is flagged by Centers for Evaluation and Information on Pharmacodependence- Addictovigilance (CEIP-A) as a drug with abuse and/or dependence potential. The Nantes CEIP-A launched a study aimed at evaluating tramadol consumption in general population. We describe the use and misuse of tramadol in outpatients.
Methods: This is a forward-looking cross-sectional epidemiological study. Data were collected through a hetero-questionnaire within a network of partner pharmacies, in Nantes area of France, from December 2011 to May 2012. Each investigator had to include the 3 first patients met in pharmacy who consumed tramadol for more than 3 months. They were trained to administer the questionnaire and obtain patient's consent.
Results: 246 outpatients were included (69.9% of female, 62 years old in average). All of them used tramadol *per os*. They consumed 260 mg of tramadol by day in average but 15 of them consumed more than 400 mg/day (1 patient consume 6000 mg/day). The large majority of the patients (94.7%) sought a pain management by the consumption of tramadol, but 7 patients (whose 5 at therapeutic dose) declared seeking a psychoactive effect (in addition to analgesic effect for 6 of them): 3 of them sought a hypnotic effect and 4 a stimulating or euphoric effect. Furthermore, 11 patients described a stimulating effect and insomnia when they use tramadol or a severe fatigue when they stop the consumption. 11 patients used from non-formal way to obtain their treatment: 6 of them used from 'doctor-shopping' or pharmaceutical nomadism, 9 of them exert pressure on the physician or on the pharmacist.
Discussion: The large majority of the patients included in this study use the tramadol according to the French SPC (Summary of Product Characteristics). Nevertheless, a misuse is observed with consumption at very high doses and/or search of positive effect (stimulant and euphoric). Even if these effects aren't described in the French SPC and begin to be reported in the literature, this study underlines the need for health practitioners to be attentive to misuse when they prescribe or dispense tramadol.
Keywords: tramadol, outpatients, misuse.

CO-100

Profile consumption of opioid maintained treatments: a national cohort study of 1074 patients from French national healthcare insurance database

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Background: Treatment of opioid dependence is based on a long-term use of buprenorphine (HBD) or methadone (MTD). The objective of this study is to describe longitudinally the consumption patterns of opioid substitution treatment (OST).
Method: Sample Generalist Beneficiaries (EGB) is a representative sample 1/97ème beneficiary of French national healthcare insurance (more than 600 000 subjects). All subjects >15 years who received a first delivery of HBD or MTD between 01/01/2007 and 31/12/2011 (no refund in the previous year) are included. Each patient was followed prospectively with the collection of all refunds OST until 01/06/2013. The 12 months retention rate in treatment was estimated.
Results: 1074 patients who received a first delivery of buprenorphine or methadone were included, mostly men (75%). The first delivery of OST was HBD ($n = 892$, 83%) with 66% princeps, and methadone ($n = 182$, 17%) with 90% syrup form. Three groups of patients were identified: first, patients with continued use of HBD exclusively ($n = 664$, 75%), secondly, the exclusive MTD group ($n = 153$, 84%) and to the end the switch group ($n = 164$) between both treatments (to MTD, $n = 144$, 16% and to HBD, $n = 20$, 11%). In the HBD group, 26% and 14% used exclusively the princeps and the generic respectively. 19% and 16% changed to the generic or princeps respectively. In the MTD group, 51% of patients used alternately syrup or capsule, syrup only 41% and 8% the capsule alone. The median durations of treatment were 219 [178–268], 321 [255–499] and 148 days [100–215] for HBD, MTD and switch group respectively ($P = 0.001$). The 12 months retention rates in the HBD, MTD and switch groups were 40%, 50% and 28% ($P < 0.001$).
Conclusion: The majority (75–84%) of opioid maintained patients used the same molecule during the first year of treatment with a 12 months rate retention significantly higher for methadone maintained patients. Finally, the switch between both treatments seems to be associated with a shorter retention in care.
Keywords: opiate substitution treatment, retention rate, buprenorphine, methadone.

CO-101

Inappropriate neuroleptic drug prescribing in nursing homes and organizational factors: a multilevel approach

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Introduction: Neuroleptic prescribing widely varies in nursing homes (NH) independently of residents' characteristics. The potential impact of NH organization on neuroleptic prescribing, and particularly inappropriate prescribing is not known. The aim of this study was to determine if the number of different general practitioners (GPs) and other facilities characteristics should influence inappropriate neuroleptic prescribing among NH residents.
Methods: This was a cross-sectional analysis of baseline characteristics of NH residents of the IQUARE study, a multicentric individually-tailored controlled trial including 6275 subjects in 175 NH in Midi-Pyrénées area. Baseline data were collected in May-July 2011 by the referent physician. These data concerns residents' characteristics, nursing home structure and internal organization and drug prescriptions in the week of resident's enrollment. The primary outcome measure was inappropriate prescribing of neuroleptic drugs in accordance with French guidelines. The analysis was done on the sub-sample of NH residents with at least one N05A prescription, and divided in appropriate/not appropriate neuroleptic prescribing. Due to the hierarchical structure of data (resident level and facility level), a multilevel binary logistic model was used.
Results: Among the 6275 NH residents (73.7% women; mean age 86 ± 8.2 years), 1532 (24.41%) had at least one prescription of neuroleptic. Of these, 513 (34.49%) had prescriptions that followed national guidelines -with regards to indication, substance and dose- and 1019 (66.51%) had an inappropriate prescribing of neuroleptic drug. Residents who lived in a nursing home with more than 30 GPs/100 beds have a higherrisk to have an inappropriate prescribing than those who live in a nursing home with <10 GPs/100 beds (Odds Ratio 1.80, 95% Confidence Interval 1.04–3.12).

Conclusion: Facility characteristics, regardless of residents' characteristics, have an influence onthe quality of the neuroleptics prescribing. This finding is of particular importance because facility characteristics are more easily modifiable than subject-related characteristics. Further studies are needed to determine the other facility characteristics affecting the appropriateness of neuroleptic prescribing.
Keywords: nursing home, neuroleptic, potentially inappropriate prescribing, elderly.

CO-102

Performance of the standardised MedDRA queries

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Objective: To evaluate the performance of Standardised MedDRA Queries (SMQs) in adverse event (AE) identification.
Methods: AE cases recorded in the French Pharmacovigilance database in 2009 were used to test four SMQs in their narrow or broad versions: agranulocytosis, demyelination, osteonecrosis, and psychosis. Free-text search performed in report narratives was used to identify a set of potential cases. These were blindly validated by two co-authors, and confirmed cases constituted the reference set for this study. Sensitivity (Se), Specificity (Sp), Positive Predictive Value (PPV), and Negative Predictive Value (NPV) of narrow and broad versions of each SMQ for the identification of the AEs of interest were calculated and reported as proportions with 95% exact confidence interval [95% CI].
Results: From the 20 830 AE cases reported in 2009, 337 confirmed cases of agranulocytosis, 17 of demyelination, 52 of osteonecrosis and 230 cases of psychosis could be included in the reference set. For agranulocytosis, Se and PPV were respectively 62.9% [57.5–68.1] and 46.8% [42.1–51.5] for narrow SMQ vs. 63.1% [57.7–68.3] and 43.6% [39.2–48.2] for broad SMQ. For demyelination, Se and PPV were respectively 88.2% [63.6–98.5] and 34.1% [20.5–49.9] for narrow SMQ vs. 94.1% [71.3–99.9] and 34.8% [21.4–50.3] for broad SMQ. For osteonecrosis, Se and PPV were respectively 94.2% [84.1–98.8] and 74.2% [62.0–84.2] for narrow SMQ vs. 94.2% [84.1–98.8] and 52.7% [42.1–63.1] for broad SMQ. For psychosis, Se was and PPV were respectively 75.1% [69.0–80.6] and 87.8% [82.3–92.0] for narrow SMQ vs. 82.5% [77.0–87.2] and 61.4% [55.7–66.8] for broad SMQ. For all selected SMQs, in both narrow and broad versions, Sp and NPV were >98%.
Discussion: SMQs were created to improve the performance of MedDRA use for AE identification, yet the literature regarding their effectiveness in spontaneous reporting databases is scarce. This study showed variable performance of the selected SMQs in the French pharmacovigilance database. The performance in the present report supports the use of broad demyelination SMQ and the narrow osteonecrosis SMQ for case identification; it appears insufficient for psychosis and agranulocytosis SMQs. Further research is needed to better evaluate the effectiveness of SMQs in spontaneous reporting databases.
Keywords: Standardised MedDRA Queries, spontaneous reporting, case identification.

CO-103

Exposure to psychotropic drugs during pregnancy: a longitudinal approach in EFEMERIS database

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Introduction: Administrative healthcare databases can provide useful data on prescription drug exposure to evaluate risks of drugs during pregnancy. However, they lack information such as profile of patients exposure according to the time when risks of drug in pregnancy are possibly different according to dose and duration of exposure. Moreover, due to the high number of data involved, it is necessary to use mathematical tools to take all of them into account.
Objectives: The aim of this study was to use a new mathematical method to describe individual trajectories of exposure to psychotropic drugs during pregnancy and to identify homogeneous groups of women exposed to psychotropic drugs during pregnancy.
Method: The present study concerns pregnant women included in EFEMERIS database who gave birth to a baby between 2004 and 2010 and who received at least one dispensation of psychotropic drug during pregnancy. After calculating the number of Defined Daily Dose (DDD) by month of pregnancy and by woman, individual exposure trajectories were graphically represented. KML method, an implementation of k-means specially design to cluster longitudinal data, was used to classify pregnant women in homogeneous exposure groups.
Results: Exposure trajectories of more than 3500 women exposed to psychotropic drugs during pregnancy were studied. Four profiles were identified: a group with a low exposure during whole pregnancy (75.5% of the women), a group with a decreasing exposure during the first trimester of pregnancy and a low exposure during late pregnancy (17.5%), a group with a moderate but stable exposure during whole pregnancy (6.2%) and a group with a constant high level of exposure (0.8%). Clusterization was performed for each of the 4 classes of psychotropic drugs (anxiolytics/hypnotics, antidepressants, antipsychotics and antiepileptics) showing large differences between these groups.
Discussion: This new mathematical approach elicits a more precise description of psychotropic exposed women all along their pregnancy. These groups of women with homogeneous psychotropic exposure profile will be used to investigate the relationship between exposure to psychotropic drugs in utero and abnormalities of psychomotor development at 9 and 24 months of age.
Keywords: pregnancy, psychotropic drugs, exposure, longitudinal approach.

CO-104

Levetiracetam and breastfeeding: long-term follow-up

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Objectives: Although levetiracetam (LEV) is extensively transferred into breast milk, only low serum concentrations are usually found in breastfed babies. In the literature, among 19 breastfed infants from mothers treated with LEV, only one experienced hypotonia and poor breastfeeding when LEV was added to the maternal phenytoin and valproic acid treatment. Our aim is to report our experience on LEV and breastfeeding.
Material and methods: All LEV-treated mothers who contacted Lyon Pharmacovigilance Center between 2004 and 2013 before initiating breastfeeding, and finally decided to breastfeed their infant for at least 7 days, were included. Health professionals and/or patients were systematically contacted to collect further data on growth and general health of the newborns.
Results: Seventeen women (18 pregnancies with 3 premature births) were included. All but one were treated for epilepsy at a mean LEV daily dose of 1587 mg (500–3000) and 8 were also treated with other antiepileptic drugs. Breastfeeding with LEV was initiated immediately after birth in 14 patients, and 5–57 days after delivery in the others. The mean duration of breastfeeding was 65 days (10–224) and 13 newborns were exclusively breastfed. After a mean follow-up of 18 months (3 weeks–6 years), all but 2 infants had normal development and weight gain. One baby was hospitalized during 2 days at the age of 25 days for sedation, vomiting and weight loss, with rapid improvement after stopping breastfeeding. His mother received daily LEV (3000 mg) and topiramate (200 mg). In the other baby, breastfeeding was stopped after 15 days because of insufficient mother's milk output associated with poor infant weight gain. Interestingly, 6 additional mothers stopped or reduced breastfeeding because of insufficient lactation. Finally, the serum LEV level measured in a 10-day asymptomatic baby breastfed from delivery (maternal LEV dose: 3000 mg) was 2.10 mg/L (therapeutic range: 5.80–17.80).
Discussion: Our data confirm that LEV is probably safe during breastfeeding. However, until more data are available and considering the two cases of hypotonia or sedation with loss of weight, careful clinical monitoring is still required. In addition, a potential deleterious effect of LEV on milk production cannot be ruled out.
Keywords: levetiracetam, breastfeeding.

CO-105

Unlicensed and off-label drug used: a prospective study in French neonatal intensive care units

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Background and aim: Unlicensed and off-label prescription is widespread. The incidence of this practice in large cohort of newborns in France has not been reported. The aim of this study was to determine the extent and nature of unlicensed and off-label prescribing in French hospitalized neonates.
Subjects and methods: A 1-year prospective study was conducted on drugs administered in two Neonatal Intensive Care Units of French University Hospitals. Drug labeling status was determined according to French summary of products characteristics in Theriaque 2013.
Results: A total of 8891 prescriptions for 142 different products were prescribed to 910 hospitalized neonates throughout the study period. Overall 64.7% (CI 95%: 63.7–65.7) of prescriptions were either unlicensed or off-label; 59.5% (CI 95%: 58.5–60.5) were off-label and 5.2% (CI 95%: 4.7–5.7) were unlicensed. 94.8% (CI 95%: 93.3–96.3) of neonates and more than 98% (CI 95%: 96.9–99.1) of preterm neonates received at least one unlicensed or off-label prescriptions. Antibacterial, central nervous system drugs and vitamins were the most commonly prescribed. Age was the most common reason for off-label prescription (58.8% (CI 95%: 57.8–59.8)).The multivariate analysis suggests that small gestational age (OR = 0.10695% CI (0.045–0.254), $P < 0.0003$) and length of hospital stay (OR = 1.1 95% CI (1.04–1.16), $P < 0.0003$) increase off-label or unlicensed drug use.
Conclusion: Unlicensed and off-label drug usage remains common in neonates. This high rate calls for action from medical professionals, institutions and pharmaceutical industry in order to emphasize effective and safe pharmacotherapy in this susceptible population.
Keywords: drug labeling, drug utilization, off-label Use, intensive care units, neonatal, newborns, infant premature, clinical trial.

CO-106

The relationship between adverse drug reactions and off-label or unlicensed drug use in paediatric in-patients

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Objective: The aim was to provide a systematic review of the results of studies performed in different settings worldwide studying the relationship between adverse drug reactions and off-label or unlicensed drug use in paediatric in-patients.
Method: A literature review of studies concerning the relationship between adverse drug reactions and off-label or unlicensed drug use in children in hospitalized settings was performed using Medline (1966–November 2013), Embase (1980–November 2013) and Cochrane Library. The references of all the studies identified were searched for additional, relevant studies.
Result: We found 2 meta-analysis including 30 studies (Pandolfini 2005) and 52 studies (Cuzzolin 2006) and 3 primary studies (Tunner 1999, Santos 2008 et Bellis 2013). All studies involved paediatric hospital wards and neonatal hospital

wards. In general, off-label/unlicensed prescription rates ranged from 11% to 80%, and higher rates were found in younger vs. older patients. On the paediatric hospital wards, offlabel/unlicensed prescriptions ranged from 16% to 62%. In the neonatal wards, rates ranged from 55% to 80%. The percentage of unlicensed and/or off-label prescriptions involved in an adverse drug reaction ranged between 23% and 60%. Only 2 studies identified relative risk (RR). Off-label drug use was significantly associated with adverse drug reactions (unadjusted relative risk 2.44; 95% CI 2.12, 2.89) in a prospective study of a paediatric reference hospital in Brazil (Santos2008) and RR 2.25 (95% CI 2.82–4.44) in a nested control study including 10 699 prescriptions of 1388 patients (Bellis2013). A prospective and multicenter (EREMI study) has been started in France to identify the relationship between ADRs and unlicensed and/or off-label prescriptions in several paediatric wards.
Keywords: adverse drug reactions, hospitalized children, off-label drugs, unlicensed drugs, prospective trials.

CO-107
Paediatric adverse drug reactions: a review of spontaneous reports registered by a Regional PharmacoVigilance Center
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Objectives: The aim of the present study was to analyze spontaneous reported Adverse Drug Reactions (ADRs) in children to Regional Pharmacovigilance center during a 27-year period and to describe their profile according to age groups.
Methods: A retrospective analysis of ADRs reports was performed involving ADRs registered in subjects.
Results: A total of 2164 cases concerning 3438 ADRs were identified and included in the study. The number of notifications increased all around the period of the study (119 from 1985 to 1989; 241 from 1990 to 1994, 515 from 2005 to 2009 and 460 from 2010 until 2012). Fifty-one percent (51%, $n = 1109$) of ADRs were observed in males. Number of reports by age group were 31 (1.4%) for neonates, 420 (19.4%) for infants, 953 (44.1%) for children and 758 (35.1%) for adolescents. ADRs were serious in 793 reports (37%). Whatever age group, the most frequent ADRs reported were skin reactions (23%), general disorders (15.3%) and nervous system disorders (14.4%). Among ATC drug classification, whatever age groups, antibacterials were the most reported. In children <12 years old, antibacterials were followed by vaccines and antineoplastic drugs. In contrast, in adolescents, after antibacterials (14.7%), the most reported drugs were psycholeptics (10.9%) and analgesics (9.5%).
Conclusions: The increasing reporting rate in the paediatric population demonstrates real concerns for children taking medicines. Moreover, the distinct pattern of drugs reported in each age group of this study highlights the importance of grouping neonates, infants, children and adolescents separately.
Keywords: adverse drug reaction, paediatric, age group, drugs, pharmacovigilance database.

APNET

CO-108
Effective absorption of acetylsalicylic acid in patients with short bowel syndrome
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Background: Patients suffering from short bowel syndrome (SBS) frequently require anti-platelet therapy. Resection of the bowel is likely to modify the absorption and first-pass effect of drugs. No data on the absorption and pharmacodynamic efficacy of the cardiovascular dose of aspirin (75–160 mg) in these patients has been published.
Methods: The pharmacodynamic (PD) efficacy of a low-dose of aspirin was assessed in 10 ten consecutive SBS patients, both 1 and 24 h after administration (peak and trough value respectively). The primary criterion was inhibition of platelet aggregation, as assessed by Light Transmission Aggregometry (LTA), triggered with 0.5 mg/mL arachidonic acid (AA). Biological efficacy of aspirin was also evaluated by serum thromboxane B2 value and by Platelet Function Analyzer-100.
Results: At its peak value, aspirin had the expected efficacy, as demonstrated both by LTA and the other techniques. However, 24 h after administration, as many as 30% of patients have lost the pharmacological efficacy of their aspirin.
Conclusion: We show for the first time, that with at least 30 cm of small intestine, all patients with SBS absorb sufficient oral aspirin in a cardiovascular dose to rapidly exert the expected level of antiplatelet activity. But given only once daily, aspirin does not provide stable 24 h antiplatelet protection in 30% of patients, due to increased platelet turnover, as usually observed in patients with extensive vascular pathology, diabetes or inflammation.
Keywords: short bowel syndrome, aspirin efficacy.

CO-109
Randomized double-blind placebo-controlled clinical trial of a dietary supplement (target-1®) on professional fatigue syndrome (burnout)
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Background: The professional fatigue or burnout syndrome is the result of severe professional stress, and carries a considerable societal burden. To date, treatments have shown little evidence of efficacy. We tested a dietary supplement combining products purported to have anti-stress or anti-fatigue effects.
Methods: Double-blind randomized placebo-controlled clinical trial of a dietary supplement including casozepine, a melon extract, taurine, and Eleutherococcus senticosus (Target-1®), given daily for 12 weeks. Main outcome was difference of change between treatment groups in the 7-point BMS-10 burnout scale. Secondary outcomes included the MBI-HSS burnout scale, Beck Depression inventory, and visual analogue scales for professional and family quality of life, quality of sleep and perceived energy.
Results: After 12 weeks of treatment or placebo, the BMS-10 went from a mean of 5.0 (SD 0.5) to 2.7 (SD 0.9) in the active group ($n = 44$) vs. 4.9 (0.5) to 4.3 (0.8) in the placebo group ($n = 43$). Treatment effect size was 1.7 points (95% Confidence Interval (CI) 1.3–2.1), $P < 0.001$. Relative improvement was 34.2% (95% CI 26.9–41.5), $P < 0.001$. Initially 86% of active group patients were classified as severe/very severe burnout compared to 4% at 12 weeks. In the placebo group these figures were 78% and 44%, respectively ($P < 0.0001$ vs. active). Secondary outcomes showed similar results.
Conclusion: This dietary supplement was associated with significant improvement of the symptoms of professional stress or burnout, after 12 weeks' treatment, compared to placebo.
Keywords: stress, burnout, professional fatigue syndrome, food supplement, randomized controlled trial, placebo.

CO-110
Placebo vs. placebo to cure depression
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Background: Antidepressants are often considered to be mere placebos despite the fact that meta-analyses are able to rank them. It follows that it should also be possible to rank different placebos, which are all made of sucrose. To explore this issue, which is rather more epistemological than clinical, we designed an unusual meta-analysis to investigate whether the effects of placebo in one situation are different from the effects of placebo in another situation.
Methods: Published and unpublished studies were searched for by three reviewers on Medline, the Cochrane Library, Embase, clinicaltrials.gov, Current Controlled Trial, in bibliographies, and by mailing key organizations. The following studies in first-line treatment for major depressive disorder were considered to construct an 'evidence network': (i) randomized controlled trials (RCTs) vs. placebo on fluoxetine, venlafaxine and (ii) fluoxetine vs. venlafaxine head-to-head RCTs.
Two network meta-analyses were run to indirectly compare response and remission rates among three different placebos: (i) fluoxetine placebo, (ii) venlafaxine placebo, and (iii) venlafaxine/fluoxetine placebo (that is, placebo compared to both venlafaxine and fluoxetine). Publication biases were assessed using funnel plots and statistically tested.
Results: The three placebos were not significantly different in terms of response or remission. The antidepressant agents were significantly more efficacious than the placebos, and venlafaxine was more efficacious than fluoxetine. The funnel plots, however, showed a major publication bias.
Discussion: The presence of significant levels of publication bias indicates that we cannot even be certain of the conclusion that sucrose equals sucrose in trials of major depressive disorder. This result should remind clinicians to step back to take a more objective view when interpreting a scientific result. It is of crucial importance for their practice, far more so than ranking antidepressant efficacy.
Keywords: antidepressants, placebo, major depressive disorder, meta-analysis, publication bias.

CO-111
Obstructive sleep apnea syndrome and statin therapy: a multicenter randomized controlled trial
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Rationale: Accumulated evidence implicates oxidative stress, sympathetic activation and inflammation as key mechanisms for hypertension, endothelial dysfunction, dyslipidemia and atherosclerosis in obstructive sleep apnea syndrome (OSAS). Statins through their pleiotropic properties are able to improve lipid profile, reduce inflammation and preserve endothelial function and might modify cardiovascular outcomes related to OSAS. This study aimed to compare the effects of atorvastatin with placebo treatment on endothelial function.
Methods: This multicenter, randomized, double-blind, parallel group study compared atorvastatin 40 mg/day and placebo over 12 weeks. The primary endpoint was endothelial function change from baseline at 12 weeks measured by periph-

eral arterial tone (PAT). Other endpoints included office blood pressure (BP), carotid early atherosclerosis (intima media thickness (IMT) and carotid diameters), arterial stiffness measured by pulse wave velocity (PWV) and metabolic parameters. Primary analysis was intent to treat.
Results: Fifty-one severe OSA patients (apnea + hypopnea index (AHI): 44.4 ± 16.2/h) were randomized. Key demographics data for the study population were: age 54 ± 11 years, 21.6% female, BMI 28.5 ± 4.5 kg/m^2. At endpoint, mean difference in endothelial function (PAT) between atorvastatin and placebo groups was 0.008 (−0.29; 0.28) (P = 0.98). Total cholesterol (mean difference: −0.89 g/L (−1.50; −0.27), P < 0.0001) and LDL cholesterol (mean difference: −0.65 g/L (−0.96; −0.34), P < 0.0001) significantly improved with atorvastatin, after 12 weeks of treatment. Systolic BP significantly decreased with atorvastatin (mean difference: −6.34 mmHg (−12.68; −0.01), P = 0.05) whereas carotid IMT (mean difference: 5.34 µm (−51.34; 62.02), P = 0.85), diameters and PWV (mean difference: 0.54 (−0.45; 1.52), P = 0.19) were unchanged compared to the placebo group.
Conclusions: In OSA patients, 12 weeks of atorvastatin treatment neither improved endothelial function nor reduced early signs of atherosclerosis although it lowered blood pressure and improved lipid profile. Trial registration number: NCT00669695.
Keywords: obstructive sleep apnea syndrome, statin, endothelial function.

CO-112
A randomized multicenter trial to assess the efficacy and safety of retrievable vena cava filter for the prevention of pulmonary embolism recurrences
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Background: Vena cava filter insertion in patients with an acute proximal deep vein thrombosis(DVT) with or without pulmonary embolism(PE) is associated with a lower risk of PE at short-term but an increase risk of DVT at long term [1,2]. In this way, retrievable filter may be used during the high-risk period of PE recurrences and then removed to avoid the late harmful effect, but this strategy was never assessed.
Methods: We conducted a multicenter open randomized study with a blind evaluation comparing retrievable vena cava filter (ALN) for 3 months with no filter insertion in patients receiving anticoagulant for an acute symptomatic PE. Patients were eligible if considering at high risk of PE recurrences (concomitant thrombosis of the lower limb AND age over 75, right ventricular dysfunction, active cancer, bilateral and/or ilio-caval DVT, or cardiorespiratory insufficiency). A systematic retrieval of filter was planned at 3 months and both groups were followed-up for 3 additional months. The primary efficacy outcome was recurrence of PE at 3 months. Secondary outcomes were PE, DVT, major bleeding and overall mortality up to 6 months.
Results: The study recruited 399 patients from August 2006 to July 2012: 200 given filter and 199 given no-filter. Median age was 76 years, 35% had a history of DVT or PE, 25% had a cancer. Recurrence of PE at 3 months occurred in 6 patients (3.0%) in the filter group compared to 3 patients (1.5%) in the no-filter group, RR = 2.00 (0.51–7.89). Filter removal was attempted in 165 patients and successful for 152. At 6 months, no difference was found on PE recurrences (7 vs. 4), DVT (1 vs. 2) or major bleeding (13 vs. 15) respectively. Twenty-one patient (10.6%) died in the filter group and 15 (7.5%) in the no-filter group, RR = 1.40 (0.74–2.64).
Conclusions: In the lack of contra-indication to anticoagulation, these results do not plead for retrieval vena cava filter insertion in patients with an acute PE.
Fundings: PHRC (French Health Ministry), Fondation de l'Avenir, Fondation de France.
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Keywords: randomized clinical trial, pulmonary embolism, vena cava filter.

COMMUNICATIONS EN PHARMACOLOGIE

CO-113
PTP1B gene deletion or pharmacological inhibition improves glucose metabolism and limits cardiovascular dysfunction in experimental septic shock
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Objective: Hyperglycemia is a common feature of septic patient and has been associated with poor outcome and higher mortality. Results of a large randomised controlled trial showed clear mortality benefits from intensive insulin therapy for patients in intensive care units. A major problem in achieving tight glycemic control with insulin is hypoglycaemia with an increased risk of death. Protein Tyrosine Phosphatase 1B (PTP1B) is a negative regulator of insulin signaling. Recently we showed that PTP1B gene deletion improves cardiovascular dysfunction during endotoxemia. However, the effect of PTP1B inhibition on glucose metabolism and cardiovascular insulin resistance during sepsis is unknown.
Materials and methods: To assess the potential effects of PTP1B inhibition, we developed a Cecal Ligation and Puncture (CLP) model of sepsis which is known to present metabolic disorders similar to those of clinical sepsis.
Results: Impaired glucose metabolism was found in mice 16 h after CLP induction as shown by the disruption of glucose intake and insulin response during glucose (GTT) and insulin (ITT) tolerance tests. PTP1B−/− mice showed improved GTT and ITT (GTT 120 min: CLP WT 20.1 ± 2.1, CLP PTP1B$^{-/-}$ 12.5 ± 2.4 mM, P < 0.001; ITT 120 min: CLP WT 2.4 ± 0.2, CLP PTP1B$^{-/-}$ 0.60 ± 0.03 mM, P < 0.001). Moreover, the limited increase of insulinemia in CLP PTP1B−/− mice emphasizes the improvement of glucose metabolism.
Insulin- and flow-mediated dilatation assessed in isolated-perfused mesenteric arteries was abolished during CLP and was improved by *ex vivo* PTP1B inhibition (% dilatation: Ins10^{-5}M, Normal values CLP 7 ± 2, CLP + PTP1Bi 18 ± 4%, P < 0.01; 200 µl/min flow, Normal values CLP 1 ± 1, CLP + PTP1Bi 13 ± 3%, P < 0.01). Arteries isolated from PTP1B$^{-/-}$ mice were protected against TNF-α induced impairment of dilation to insulin (Ins10^{-5}M, WT + TNF-α 7 ± 1, PTP1B$^{-/-}$ + TNF-α 20 ± 2%). We also found that PTP1B$^{-/-}$ mice subjected to CLP had a higher survival rate compared to WT (duration of 50% survival, WT 28 h, PTP1B$^{-/-}$ 42 h, P < 0.05).
Discussion: PTP1B gene deletion or inhibition limits sepsis-induced hyperglycemia and insulin resistance, Resolution of hyperglycemia with PTP1B inhibition is associated with reduced vascular dysfunction and increased survival. PTP1B inhibition may represent a new effective strategy in the treatment of septic insulin resistance with hyperglycemia.
Keywords: sepsis, insulin resistance, hyperglycemia, cardiovascular dysfunction, PTP1B.

CO-114
Prevention of target organ damage by soluble epoxide hydrolase inhibition in a murine model of type 2 diabetes
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Objective: Epoxyeicosatrienoic acids (EETs) are eicosanoids derived from arachidonic acid, notably in endothelial cells, and display attractive metabolic, vasodilatory and anti-inflammatory properties. We demonstrated previously that inhibiting EET degradation mediated by soluble epoxide hydrolase (sEH) reduces hypertension and heart failure, and others reported that it improves glucose homeostasis in type 2 diabetes. However, the impact of such strategy on target organ damage in diabetes remains to be clarified.
Materials and methods: This study investigates the metabolic, cardiovascular and renal effects of sEH pharmacological inhibition with *t*-AUCB (10 mg/l in drinking water) administered for 8 weeks in mice subjected to a high-fat diet (HFD, 60% fat) for 16 weeks, compared to mice on control chow diet (10% fat), non-treated HFD mice and HFD mice treated with glibenclamide (GLI: 80 mg/l).
Results: Glibenclamide and *t*-AUCB similarly prevented the increased fasting glycemia in HFD mice (Control: 5.4 ± 0.2; HFD: 8.0 ± 0.8; HFD + GLI: 5.1 ± 0.3; HFD + *t*-AUCB: 5.6 ± 0.2 mM). However, only *t*-AUCB improved glucose tolerance and decreased gluconeogenesis, assessed by pyruvate tolerance test. This improvement in glucose homeostasis appeared independent to a reduction in peripheral insulin resistance but rather related to a decrease in hepatic steatosis and, as shown in isolated mouse pancreatic islets,to maintenance of insulin release under hyperglycemic conditions and to reduced endoplasmic reticulum stress. In parallel, only *t*-AUCB prevented adipose tissue activation and decreased plasma free fatty acids, triglycerides and LDL cholesterol. Moreover *t*-AUCB improved coronary function, decreased cardiac fibrosis and hypertrophy, and prevented diastolic dysfunction, as shown by echocardiography (E/A ratio; Control: 1.25 ± 0.02; HFD: 1.05 ± 0.03; HFD + GLI: 1.07 ± 0.04; HFD + *t*-AUCB: 1.23 ± 0.04) and left ventricular hemodynamics (end-diastolic pressure-volume relationship; Control: 1.9 ± 0.55; HFD: 3.8 ± 0.61; HFD + GLI: 3.1 ± 0.8; HFD + *t*-AUCB: 1.88 ± 0.2 mmHg/RVU). Finally, *t*-AUCB prevented the increase in the urinary albumine-to-creatinine ratio and thus, improved renal function notably by reducing renal inflammation.
Discussion: These results demonstrate that, beyond its glucose-lowering effect, sEH inhibition improves coronary endothelial function, reduces cardiac hypertrophy and diastolic dysfunction and prevents early kidney damage in a murine model of type 2 diabetes. This positive impact on target organ damage and metabolic homeostasis prompts sEH inhibition as a novel and valuable therapeutic perspective in type 2 diabetes.
Keywords: Type 2 diabetes, endothelium, cardiac function, soluble epoxide hydrolase, diabetic dyslipidemia, endoplasmic reticulum stress renal function.

CO-115
Inhibition of TRPC3 improves endothelial function in aorta of type II diabetic mouse
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Objective: The acute calcium influx through nonselective cation TRPC3 channels is a key signaling mechanism that stimulates the release of several endothelium-derived vasoconstrictive agents. An increased expression of TRPC3 in vascular endothelium has been proposed as one of the factors contributing to the pathogenesis of HTA. As endothelial dysfunction is also associated with type II diabetes we designed this study to evaluate the involvement of TRPC3 in this pathology.

Materials and methods: Aortic endothelial cells from young adult (20 weeks) and old (45 weeks) diabetic (*db/db)* mice (MAECs) were isolated from aortic vessel explants and cytosolic Ca^{2+} was recorded with an inverted epi-fluorescence microscope using Fura-2 probe. Expression of TRPC3 channel protein was determined by Western blotting. As a measure of endothelial function, relaxation to acetylcholine (ACh) was recorded on aortic rings constricted by phenylephrine.
Results: The expression of TRPC3 channel protein was increased by 50% in aorta of *db/db* mice, whatever their age. In parallel, NO-mediated relaxations to ACh were decreased in diabetic mice aorta compared to control mice (by 42% at 20 and by 53% at 45 week-old) while relaxation of smooth muscle cells to NO donor (sodium nitroprusside) were only decreased in 45 week-old *db/db* mice aorta. Compared to control mice, basal cytosolic calcium was increased and the amplitude of ACh-induced calcium response was decreased in *db/db* MAECs. In vessels from diabetic mice, Pyr3, a specific TRPC3 inhibitor, which had no effect in control mice at 1 μM, increased relaxation to ACh with no effect on NO donor response. Moreover, it normalized basal and stimulated abnormalities of cytosolic $[Ca^{2+}]$ in MAECs from diabetic vessels.
Conclusion: These studies indicate that TRPC3 overexpression and endothelial dysfunction coexist in vessels from diabetic mice. Moreover, pharmacological inhibition of the abnormal Ca^{2+} entry in endothelial cells through TRPC3 ameliorates calcium signaling in endothelial cells and consequently, NO-mediated relaxation.
Keywords: TRPC3, diabetes, endothelial cells, NOS3, NO, $[Ca^{2+}]$ signalling.

CO-116

Differential impact of regular exercise on pro-survival pathways and mitochondrial permeability transition pore in the myocardium of lean and obese mice

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Context: Obesity is known to abolish the ability of several cardioprotective strategies (pharmacological or not) to protect the myocardium against infarction by increasing the phosphorylation state of several pro-survival kinases (RISK and SAFE pathways). We previously showed that regular exercise is able to restore cardioprotection in obese ob/ob mice by increasing the phosphorylation state of these kinases and consequently by improving the resistance of mitochondrial permeability transition pore (mPTP) to opening at the onset of coronary artery reperfusion.
Objectives: our goal was to determine whether these beneficial adaptations develop as a pre-emptive mechanism, *i.e.*, before ischemia-reperfusion or rather specifically at reperfusion.
Methods: We investigated the RISK, SAFE and AMPK pathways as well as mitochondrial adaptations induced by regular exercise *per se, i.e.*, in the absence of ischemic conditions. Wild-type (WT) and obese (ob/ob) mice were assigned to sedentary conditions or regular treadmill exercise (1 h/day, 5 days/7, 4 weeks, 4° slope, 10–30 cm/s).
Results: Exercise increased phosphorylation of proteins such as Akt, ERK1/2, AMPK and STAT3 in WT as well as in ob/ob animals. Importantly the levels of corresponding phosphatases PTEN, MKP-3 and PP2C were decreased. In addition, SOD1 and catalase were significantly increased by exercise in both WT and ob/ob animals. We next investigated the consequences of these activations on mPTP opening. Surprisingly, regular exercise did not affect the calcium concentration required to open mPTP neither in WT nor in ob/ob animals.
Discussion: Although regular exercise enhances the phosphorylation of the pro-survival kinases, it did not induce pre-emptive mitochondrial adaptations that are known to be mandatory for cardioprotection. This activation of survival pathways needs to be combined with ischemia-reperfusion to initiate downstream signalling which converges toward mitochondria and inhibits mPTP opening at the onset of reperfusion.
Keywords: myocardial infarction, exercise, mPTP.

CO-117

ARA290 as a booster of endothelial progenitor cell transplantation: towards a new strategy in the treatment of chronic peripheral ischemia?

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Objectives: Endothelial colony-forming cells (ECFC) are promising candidates for cell therapy of ischemic diseases. Erythropoietin has been described to act in a synergistic way with ECFC transplantation after transient focal cerebral ischemia in rats, but clinical use of EPO in ischemic diseases is controversial. ARA290 is a non-erythropoietic EPO derivative which only binds to the cytoprotective receptor complex (EPOR2-CD131). In this work, we characterized the ARA290 properties on cultured ECFC. Secondly, we investigated the efficacy of combination of ARA290 administration and ECFC transplantation in a mouse model of chronic hindlimb ischemia.
Material and methods: *In vitro,* proliferation and protective properties against oxidative stress were assessed by ELISA. Migration was evaluated by the wound healing test.
In vivo, 24 mice were exposed to unilateral hindlimb ischemia. 24 h later, a single injection/transplantation (ARA290 and/or ECFC) was performed. Ambulatory impairment and perfusion ratio (ispi/contra) were evaluated until day 28. *In vivo* apoptosis (D3) and transplanted cells homing (3 h) to ischemic hindlimb were assessed by SPECT/CT.
Results: *In vitro,* ARA290 enhanced BrdU incorporation (138 ± 11% of control; $P < 0.01$) and decreased LDH release after oxidative stress (69 ± 11% of control; $P < 0.01$). Area recovery in the wound healing test was dose-dependently improved in ARA290 treated cells (until 180 ± 4% of control; $P < 0.001$).
In vivo, compared toECFC treated group: animals tended to have a reduced ambulatory impairment score and short-term (D7) perfusion recovery was increased in ARA290/ECFC treated group (59 ± 3% vs. 48 ± 1; $P < 0.001$); ^{99m}Tc-AnnexinV binding to damaged tissues was significantly reduced (−32%; $P < 0.05$) and ^{99m}Tc-labelled ECFC homing in ischemic hindlimb was increased in ARA290/ECFC treated group (+55%; $P < 0.01$).
Discussion: Taken together, these results suggest that the activation of the receptor complex (EPOR2-CD131) boosts ECFC regenerative properties *in vitro and in vivo*. The enhancement of transplanted cells homing, and protective properties against apoptosis (*in vivo*) and oxydative stress (*in vitro*) should contribute to early perfusion recovery. This work is the first report suggesting that ARA290, ongoing phase 2 clinical trial candidate for small fiber neuropathy in diabetes, could enhance therapeutic benefit of ECFC transplantation in chronic peripheral ischemia.
Keywords: angiogenesis, CD131, endothelial progenitor cell therapy, erythropoietin.

CO-118

Peripheral microcirculation improvement by central sympathetic nervous system modulation in obese rats with metabolic syndrome

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Objectives: Cardiovascular and metabolic risk factors that characterize the metabolic syndrome (MS), including high blood pressure, obesity and glucose intolerance, are accompanied by sympathetic hyperactivity. In this study, we investigated the effects of a chronic oral antihypertensive treatment using centrally-acting sympatho-inhibitory drugs on the peripheral microvascular dysfunction in rats under long-term high-fat diet.
Methods: Fifty male adult Wistar rats were maintained under normal diet (CON + VEH, $n = 10$) or high-fat diet (HFD + VEH, $n = 40$) during 20 weeks. Thereafter, the HFD group received oral clonidine (HFD + CLO, 0.1 mg/kg), LNP599 (HFD + LNP599, 20 mg/kg) or vehicle (HFD + VEI). Systolic blood pressure (SBP) was evaluated by photo-plethysmography during the long-term treatment and the peripheral microcirculation flow was evaluated by Laser Speckle contrast Imaging (Perimed).
Results: There was an increase in SBP and heart rate in the HFD + VEH group (166 ± 4 mmHg, $P < 0.05$ and 371 ± 7 bpm, $P < 0.05$, respectively) compared to the CON + VEH group (141 ± 3 mmHg and 330 ± 4 bpm, respectively). The HFD + VEH group also presented a decrease in blood perfusion (arbitrary perfusion units, PU) in the gracilis muscle (97 PU). Chronic treatment with both clonidine and LNP599 lowered SBP to control values and increased blood flow perfusion (HFD + CLO: 150 PU and HFD + LNP599: 130 PU, $P < 0.05$ respectively) compared to control group (100 PU). Topic administration of Ach (100 mM) on gracilis muscle was used to assess the periperal microvascular function in HFD animals. Endothetial-dependent vasodilation response to Ach was significantly increased in HFD + CLO group (57%) and in HFD + LNP599 group (48%) when compared to non-treated group (45%).
Conclusions: These results suggest that the modulation of sympathetic activity results in a simultaneous reduction of SBP accompanied by a reversion of capillary rarefaction and improvement of endothelial function in the skeletal muscle of MS-induced rats.
Keywords: microcirculation, sympatho-inhibitory drugs, laser speckle imaging.

CO-119

Correlation study between [123I]-FP-CIT binding and nociceptive threshold in Parkinson's disease

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Objectives: Pain is a non-motor symptom frequently reported in Parkinson disease (PD). Previous studies showed abnormal activations of nociceptive brain areas and a lower pain threshold partially normalized by levodopa in PD patients. The aim of this study was to investigate a putative correlation between striatal and extrastriatal dopaminergic system and pain threshold in PD patients.
Patients and methods: We included 25 PD patients with various intensity of painful sensations rated on a 100 mm-Visual Analogic Scale (VAS) (from 0 to 86). Subjective heat pain threshold (thermotest) and motor examination (UPDRS part III) were performed after dopamine withdrawal of 12 h. Each patient had a SPECT imaging with [^{123}I]-FP-CIT binding dopamine transporters. We performed statistical correlations between [^{123}I]-FP-CIT binding and subjective pain threshold values using a simple linear regression model for striatal uptake and using a voxel-based approach for extrastriatal uptake. The covariables were age, gender, duration of PD and UPDRS motor score.
Results: The mean age of PD patients was 64.1 ± 6.8 years, with a mean duration of PD of 9.2 ± 5.2 years, and a mean UPDRS motor score of 26.3 ± 8.0. The striatal analysis revealed significant negative correlations between [^{123}I]-FP-CIT binding and age ($P = 0.02$), duration of PD ($P = 0.0002$) and UPDRS motor score ($P = 0.006$) but no significant correlation was found with pain threshold. The extrastriatal analysis showed a significant positive correlation between [^{123}I]-FP-CIT binding and subjective heat pain threshold in the left posterior cingulate cortex (PCC) ($P < 0.001$) and a significant negative correlation in the left insula ($P < 0.001$).
Discussion: These results suggest that pain perception abnormalities in PD are not directly related with striatal dopaminergic dysfunction. However, painful sensations could be related with extrastriatal dopaminergic dysfunction, with an imbalance between the sensory (insula) and the affective (PCC) cerebral nociceptive pathways.
Keywords: Parkinson's disease, pain, [123I]-FP-CIT.

CO-120

Erythropoietin improves nociception and restores skin protection against pressure-induced ulcer in small-fiber neuropathy mouse model

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An increased risk of pressure-induced ulcers (PUs) is common in patients with sensory neuropathy. The recombinant human erythropoietin (rhEPO) protects skin against PUs during long-term diabetes with neurovascular complications. In this report, we aimed to determine whether rhEPO could prevent PUs formation in mice, expressing only a sensory small-fiber neuropathy. To investigate involvement of sensory small-nerve fiber in PUs development, a reversible small-fiber neuropathy mice model induced by resiniferatoxin (RTX) was developed. Seven days after RTX administration, mice expressed thermal hypoalgesia, neuropeptides (CGRP, SP) depletion in sensory neurons without nerve degeneration, and showed a visually and histologically larger stage 2 PUs. All effect induced by RTX at day 7 were normalized 3 weeks later. RTX and Control mice received either no treatment or systematic rhEPO 1 day before RTX administration and each other day during 7 days (3000 UI/kg intraperitoneally). At day 7 in both groups, rhEPO treatment impairs endothelium-vasodilation responses. In RTX mice at day 7, rhEPO restored skin capacity to protect against pressure/ischemia injury and prevented thermal and mechanical hypoalgesia, CGRP depletion in intraepidermal nerve fiber (IENFs) and improved SP depletion in IENFs. In summary, RTX increased PUs development by a functional impairment of cutaneous small-fibers with neuropeptides depletion. The endothelial damage induced by rhEPO is not harmful to the skin protection against pressure-induced ulcer. Our finding that rhEPO treatment prevents nociceptive behaviours, neuropeptides depletion with a skin protection against pressure-induced ulcers in neuropathic mice, encourages evaluation of therapeutic potential in preventing neuropathic ulcers.

Keywords: pressure ulcer, erythropoïétine, small-fiber neuropathy, nociception, neuropeptides.

CO-121

Agomelatine showed antihyperalgesic properties in a rat model of neuropathic pain and additive interaction with gabapentin

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Introduction: Antidepressants are widely used to manage neuropathic pain syndromes. Agomelatine is a new antidepressant acting as melatonin agonist (MT_1 and $MT_{2)}$ and $5\text{-}HT_{2C}/5\text{-}HT_{2B}$ antagonist. Literature shows that melatonin as well as $5\text{-}HT_{2C}/5\text{-}HT_{2B}$ receptors are involved in the control of nociceptive messages in both neuropathic and visceral pain models. Here we evaluate the antihyperalgesic effect of agomelatine compared to gabapentin given acutely as well as the effect of their combination in a rat model of neuropathic pain.

Methods: Neuropathy was induced in adult Sprague-Dawley rats by loose ligation of the right sciatic nerve and mechanical hyperalgesia was evaluated by the paw pressure test. Fifteen days after surgical procedure, post-operative thresholds were determined and vehicle and drugs intraperitoneally administered. Treatment groups were planned as agomelatine (5, 10, 20 and 45 mg/kg), gabapentin (12.5, 25, 50 and 100 mg/kg) and vehicle (hydroxyethylcellulose 1%). Vocalization thresholds were measured 15, 30, 45, 60, 90 and 120 min after drug administrations. The antihyperalgesic activity induced by agomelatine and gabapentin combination and the type of interaction was determined by using the isobolographic analysis.

Results: Agomelatine (20 and 45 mg/kg) significantly increased vocalization thresholds in a dose-dependent manner, between 15 and 60 min after its injection ($P < 0.001$), compared to the vehicle treated group. The maximal effect (at 15 min) reached almost 80% of predrug values. Gabapentin significantly and dose-dependently increased vocalization thresholds, between 60 and 90 min compared to the vehicle treated group. The maximal effect (at 60 min) reached 73% of predrug values. The ED_{25} values associated with single administration of agomelatine and gabapentin were 16.1 mg/kg and 15.6 mg/kg, respectively. Coadministration of agomelatine and gabapentin induced an additive antihyperalgesic interaction, allowing to decrease doses of both compounds and to reach the same effect that those obtained with 3 fold higher doses of each drug itself.

Conclusion: Acute administration of agomelatine clearly demonstrates antihyperalgesic effect in a well-established animal model of neuropathic pain, without any perceptible behavioral changes. Interestingly an additive antihyperalgesic effect was observed after its combination with gabapentin. Agomelatine could be a potential new pharmacological strategy for the management of neuropathic pain in humans.

Keywords: neuropathic pain, antidepressant, agomelatine, gabapentin, isobolographic analysis.

CO-122

Sweet's syndrome: a 5-case report from a clinical trial on acute myeloid leukemia

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Introduction: Sweet's syndrome (SS) is an acute febrile neutrophilic dermatosis characterized by a sudden onset with fever, leucocytosis and tender erythematous plaques or nodules infiltrated by neutrophils. SS can be idiopathic, malignancy-associated or drug-induced. We report 5 cases issued from the same clinical trial involving patients with newly-diagnosed acute myeloid leukemia (AML).

Observations: The patients received the following induction chemotherapy: I.V doxorubicin (60 mg/m² D1-3, 35 mg/m² D8-9), I.V cytarabine (500 mg/m² D1-3 and 2000 mg/m² D8-10) associated with the I.V G-CSF filgrastim (5 µg/kg/day D1–10).

A 45-year-old male subject, with SS affecting 30% of his body surface area (trunk, arms and thighs), induction latency 1st dose: 7 days.

A 55-year-old male subject with SS of neck, face, ears and upper limbs, induction latency 1st dose: 7 days.

A 53-year-old female subject with SS, induction latency 1st dose: 17 days, filgrastim was stopped 7 days before.

A 41-year-old female subject with SS, induction latency 1st dose: 8 days, 6 days with filgrastim.

A 32-year-old male subject with SS of hands and legs associated with pulmonary infection, induction latency 1st dose: 7 days.

Most of SS occurred within 7 days after the first induction day. SS was confirmed by cutaneous biopsy for all cases. Filgrastim was suspected and stopped while doxorubicin and cytarabine were continued. All patients recovered spontaneously or after corticosteroids treatment. For 2 patients, filgrastim was reintroduced with shorter duration and SS did not reoccur.

Discussion: SS is a rare adverse event (<0.01%). In this trial, SS was reported in approximately 1% of patients. Most of drug-induced SSs are related to granulocyte-colony stimulating factor (G-CSF) however SS is also associated with AML. Distinguishing the role of AML from drug in the occurrence of SS is not easy but regarding the timeframe, our cases are compatible with a filgrastim-induced SS. G-CSF induces neutrophils proliferation that can potentially lead to neutrophil skin accumulation explaining the SS. Filgrastim was reintroduced with a lower total dose in two patients with no recurrence. A G-CSF dose-dependent effect has already been suggested in causing Sweet's syndrome.

Keywords: Sweet's Syndrome, filgrastim, acute myeloid leukemia.

CO-123

Clinical characteristics associated with cardiovascular toxicity of antiangiogenic agents in oncology

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Objective: Antiangiogenics are a new therapeutic class widely used in medical oncology in advanced setting. Potentially life-threatening cardiovascular side effects are associated with these medications such as hypertension, proteinuria, renal and cardiac failures (RF, CF), arterial or venous thrombotic events (ATE, VTE) or haemorrhages. There are no validated clinical or biological predictive factors helping to better identify patients at risk of cardiovascular toxicities during antiangiogenics exposure. The aim of this study was to evaluate the impact of initial cardiovascular comorbidities on cardiovascular side effects in a cohort of patients treated with antiangiogenics.

Methods: 439 patients receiving antiangiogenic drugs for metastatic cancer were included in two teaching hospitals inFrance. Antiangiogenics included biotherapies (bevacizumab, aflibercept) and oral multikinase inhibitors (sunitinib, sorafenib, pazopanib, investigational drugs with antiangiogenic activity). Cardiovascular events were prospectively collected and graded using Common Toxicity Criteria of Adverse Events v3 definitions (hypertension, proteinuria, RF, CF, ATE, VTE). We retrospectively assessed in univariate and multivariate analysis associations between these side effects and initial cardiovascular comorbidities including pre-existing hypertension, diabetes, dyslipidemia, personal history of renal or cardiac failure, arterio-veinous thrombotic events and tobacco exposure. Impact of age, body mass index, initial performance status, site of primary cancer, type of antiangiogenic treatment were also investigated.

Results: Incidence of all grade toxicities were for each cardiovascular side effect: hypertension 40.6% ($N = 178$), proteinuria 17.8% ($N = 78$), RF 5.0% ($N = 22$), CF 2.3% ($N = 10$), ATE 2.1% ($N = 9$), VTE 3.7% ($N = 16$). In multivariate analysis, association between side effect and cardiovascular comorbidities or clinical variables were the following:: hypertension was associated with initial performance status (PS0 vs. PS1 or 2 OR = 1.89, IC95% 1.16–3.05, $P = 0.010$) and history of hypertension (OR = 1.69, IC95% 1.01–2.81, $P = 0.045$); proteinuria with history of hypertension (OR = 1.90, IC95% 1.09–3.26, $P = 0.022$); RF with history of hypertension (OR = 7.6, IC95% 2.7–22.2, $P < 0.001$). No investigated parameters were associated with ATE and VTE in multivariate analysis.

Conclusion: Incidence of all grade cardiovascular side effects was higher than expected in our population. The main predictive factors of cardiovascular toxicities in this study were prior history of hypertension and initial good performance status. These results need to be confirmed by a prospective validation study.

Keywords: antiangiogenics, cancer, side effects.

CO-124

Pseudo-gout induced by zoledronic acid: a case report, review of the literature and French pharmacovigilance database

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Introduction: Pseudo-gout is characterized by acute inflammation or synovitis involving one or more jointsis. It is linked to calcium pyrophosphate deposition. Zoledronic acid (Aclasta®; Zometa®) is the most potent bisphosphonate with concentration in bone >100-fold of plasma. Indications consist in bone complications of cancer, hypercalcemia of malignancy, osteoporosis and Paget disease.

Observation: A 79-year-old woman, with antecedent of lupus treated by prednisolone and hydroxychloroquine, started first infusion of Aclasta® for osteoporosis. On the same day, she developed fever and widespread pain which persisted on the following 2 days. Five days later, she experienced painful arthritis in several joints: fingers, wrist, cervical and knees. PCR (normal before) raised to 95 mg/l., cryoglobulinemia was negative, complement, Vit D, PTH, uric acid were normal. No antinuclear antibodies. Serum calcium dropped from 2.30 mM before infusion to 2.02 mmol/17 days after infusion. X-ray of the wrist and the hand revealed typical signs of pseudo-gout (Thin opaque border at a short distance of bone contours). The outcome was favourable in 1 month under increase of corticoid dosage.

Discussion: This is the first case report of pseudo-gout with zoledronic acid, four other cases are reported with other bisphosphonates in the literature. As it was hypothesized formerly, the acute inflammatory reaction could have induced synovial inflammation associated with the decrease of serum calcium, that may have contributed to mobilize calcium crystals. After a search in the pharmacovigilance database, sixteen cases of severe arthralgia and swelling of the joints with Aclasta® were found and four cases with Zometa®. In all cases are noted occurrence within 1–5 days after the first infusion, involvement of several joints (mainly knees and wrist), severe functional disability and improvement of symtomatology after 1–4 weeks. Diagnosis of pseudo-gout was evoked in only two cases.
Pseudo-gout is not found in the adverse event section of the SCP; knowledge of this could lead clinician to research it and give appropriate treatment.
Keywords: zoledronic acid, pseudo-gout.

CO-125

Characteristics of adverse drug reactions-induced hospital readmission in a post Emergency Hospital Unit

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Purpose: According to several studies, Adverse Drug Reactions (ADRs) leading to hospital admission was estimated to 3.6–21.7% [1].Despite its importance in terms of patients care, re-admission to hospital due to ADRs remains less documented. The aim of our study was to investigate the main characteristics of re-admission for ADRs
Methods: We undertook a retrospective study during 2 years (2011–2012) in the Post-Emergency Unit of Toulouse University Hospital. We selected all unplanned hospitalization for acute disease and included all cases of patients admitted twice or more. Characteristics of drug-induced ADRs were assessed according to appropriate use or not [2].
Results: Out of the 197 readmitted patients, 71 was related to ADRs (3.6%) corresponding to 17.8‰ patient-year. Mean age was 82.3 years and 67% were women. The most frequent ADRs found were vascular (n = 41, 18.4%), gastrointestinal (n = 28, 12.6%), cardiac (n = 28, 12.6%), neurologic (n = 26, 11.7%), metabolic (n = 26, 11.7%) and psychiatric (n = 24, 10.8%). The drugs mainly involved were psychoactive (psycholeptics, psychoanaleptics and analgesics), cardiovascular (diuretics, agents acting on the renin-angiotensin system), digestive (drugs for acid related disorders, laxatives) or antithrombotic agents. The context of occurrence of ADRs was related to inappropriate drug prescription in 56% of cases: drug overuse for 27% (n = 31), misuse for 22% (n = 26) and underuse for 7% (n = 8). Actions for managing of ADRs were drug withdrawal (n = 68, 59%), dose modification (n = 18, 15%), substitution by other drugs (n = 5, 4%), addition of a new drug (n = 7, 6%) or drug continuation (n = 12, 10%). A total of 24 patients were admitted twice and 2 patients admitted three times for the same ADR. For 22 patients, the same drugs were involved.
Discussion: Our data show hospital readmission was due to ADRs in 3.6% of cases. In 1.1% of cases, the same couple 'drug-ADR' was involved. Furtheremore, in 56% of cases, repeat admission are related to an inappropriate drug prescription.
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Keywords: repeat admission, adverse drug reaction, inappropriate prescription.

CO-126

Metformine associated lactic acidosis: impact of an early diagnosis procedure on mortality

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Objective: Metformine-associated lactic acidosis (MALA) is a rare and serious adverse effect (AE) with a mortality rate of up to 50%. The diagnosis is often delayed as clinical symptoms are atypical and as the pH and lactates assay is not part of the initial checklist on emergency admission. The aim of this work was to assess the impact of an early MALA diagnosis procedure (MALA-DP) on mortality rate.
Methods: An early MALA-DP is based on the assay of pH, lactates and metformine in all diabetics with metformine as soon as the patient is admitted to the emergency department. A before-after study was performed, comparing cases from an experimental group (EG) receiving the MALA-DP (7/2012–6/2013) with cases from a control group (CG) without the MALA-DP (1/2011–6/2012). MALA was defined as pH ≤ 7.35, lactates >5 mM in patients exposed to metformin, without other common causes of lactic acidosis. The identification of MALA was carried out, using data provided by the pharmacology laboratory, the medical information system programme database (PMSI) and after a causality assessment by the pharmacovigilance staff.
Results: 12 cases of MALA were diagnosed in the EG and 22 cases in the CG; there were no significant differences in baseline patients' characteristics. The cases in the EG had a higher illness severity than those in the CG: lactates (14.2 mM vs. 8.8, P = 0.0094), creatinine (462.8 µM vs. 272.6, P = 0.03), plasma bicarbonate (7.8 mM vs. 14.3, P = 0.0016). The time up to MALA diagnosis was divided by 4 in the EG (120 ± 137 vs. 29 ± 27 min). The global mortality rate decreased by 26.5% when comparing the CG and the EG (45.5% vs. 33.3%) and by 50% in the emergency department (18% vs. 9%).
Conclusion: The study allowed to increase the awareness of physicians in the emergency unit on a very unusual AE with metformine and to demonstrate the positive impact of an early MALA-DP on mortality rate as serious MALA cases were consequently detected more quickly.
Keywords: metformine, lactic acidosis, diagnosis procedure, mortality, before-after study design.

CO-127

Injection of pharmaceutical tablets of buprenorphine: differences between original and generics

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Introduction: Misuses of buprenorphine concern around 30% of patients treated for drug detoxification in case of opioid abuse. Injecting pills that are not intended for intravenous (IV) administration may have harmful consequences particularly because of particles. The main difference between Subutex® and its generics concern the insoluble particles subsets. The aim of our work was to determine, by analysing injected solutions, if the misuses of the generics could be more dangerous than that of the Subutex®.
Injection material used was contained in Steribox® II Kit. Solutions were prepared using Subutex® 8 mg or Buprenorphine 8 mg generic as follow: tablet was dissolved in 1 mL water, mashed with the syringe's piston and filtered through a cotton pad or a Sterifilt®. Quantification of buprenorphine concentration was performed by U-HPLC, particles size distribution was performed by laser granulometry and flow cytometer.
After filtration with cotton pad, the maximal size of the particles found in solution reached 100 µm for the generic and 47 µm for the Subutex®. With Sterifilt® the maximal size of the particles were 36 µm for the generic and under the limits of the laser granulometer for the Subutex®. These results were confirmed by flow cytometer: after sterifilt® filtration, more particles >5 µm were found for the generic with regard to the princeps. 80–100% of the dose of buprenorphine contained in a tablet was found in the solution, whatever the drug and the filter used.
Extraction recovery of buprenorphine tablets was excellent and similar for both princeps and generic. These results confirm that buprenorphine remains an excellent candidate for misuse. We have highlighted the wide variation of the quantity and the size of the particles present in solution between the two drugs after cotton pad filtration. According to these preliminary results, misuse of injected buprenorphine could be more dangerous with the generic form than with the princeps, in particular in term of thromboembolic events.
Keywords: buprenorphine, misuse, injection.

CO-128

5-HT2C serotonin and H1 histamine receptor affinity and the risk of antipsychotic-induced diabetes: a pharmacodynamic-pharmacoepidemiological study in Vigibase™

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Background: Pharmacodynamic mechanisms of diabetes induced by antipsychotic drugs remained discussed. Some basic pharmacodynamic studies suggested the involvement of 5-HT1A, 5-HT2A, 5-HT2C serotonin, H1 histamine, M3 muscarinic, α1, α2 adrenergic or D2 D3 dopaminergic receptors in genesis of this Adverse Drug Reaction (ADR).
Objective: To evaluate associations between receptor affinity of neuroleptics with case reports of diabetes into Vigibase®, the WHO monitoring database of pharmacovigilance.
Methods: We estimated by multivariate logistic regression the association between reports of drug-induced diabetes into Vigibase® (between the 1st January 1994 and the 29th March 2013) and receptor affinities of 17 antipsychotics. Results were adjusted on potential confounding factors (age, gender, duration of drug marketing, exposure to hypo- and hyperglycemic drugs, characteristics of the notificator, geographic area). A case non-case study with these 17 drugs was also performed.
Results: Among the 96 170 notifications, 1801 (1.87%) involved diabetes as an ADR. A significant and positive association between 5-HT2C and H1 affinity and notifications of diabetes was found. In comparison to notifications with low 5-HT2C affinity drugs (reference), OR for developing diabetes was 0.62 (95% CI [0.42–0.93]) for average 5-HT2C affinity drugs and 3.15 (95% CI [1.29–7.67]) with high 5-HT2C affinity drugs. In comparison to notifications with low H1 affinity drugs (reference), OR for developing diabetes was 6.51 (95% CI [3.73–11.35]) for drugs with average H1 affinity and 3.34 (95% CI [1.19–9.41]) for drugs with high H1 affinity. D2 and α1 affinity were negatively associated with reports of diabetes, whereas no significant association was found for other investigated receptors (5-HT1A, 5-HT2A, M3,. α2, D3). The case non-case study found that 3 drugs were positively associated with reports of diabetes: olanzapine (ROR = 2.69), clozapine (1.50), perphenazine (1.04).
Conclusions: This work gives an example of a new method in pharmacoepidemiology: the pharmacovigilance-pharmacodynamic (PV-PD) study which allows to investigate the role of drug receptors in the mechanism of ADRs. Our data suggest the involvement of 5-HT2C and H1 receptor affinity of drugs in the mechanism of antipsychotic-induced diabetes.
Keywords: 5-HT2C, H1, Affinity, antipsychotics, diabetes.

CO-129

Does substitution of brand name medications by generics differ between pharmaco-therapeutic classes? A population-based cohort study in France

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Purpose: Substitution of brand name medications by cheaper generic alternatives allows reducing health care costs. For antiepileptic drugs (AEDs), substitution by bioequivalent generics has been linked to reports of seizure control's loss. According to a meta-analysis, seizures' increase was only found in observational studies but not in clinical studies [1]. This increase could probably be explained by anxiety of patients (also called 'anticipatory anxiety'). The aim of our study was to measure the proportion of patients switching from generics to branded drugs among users of antiepileptics compared to 2 other pharmaco-therapeutic classes (neuroleptics, beta-blockers).

Methods: We performed a prospective observational study of the switchback (i.e. from generic drug back to its branded drug) rates. We conducted a cohort study involving subjects included in the French health insurance system database from January 2009 to November 2012. The maximum follow-up duration was 44 months. Selected patients took the branded drug therapy for 60 days or more during the 90 days preceding the generic substitution. Association with gender, age (under 75 vs. 75 years or more), treatment characteristics, number of switches between generics, type of prescriber when switching were estimated using Relative Risk. Descriptive statistics and the Cox proportional hazard regressions used SAS 9.3.

Results: 6330 patients were included in the cohort, 1903 with antiepileptics, 2342 with neuroleptics and 2085 with beta-blockers. Rate of switchback was estimated to 69% ($n = 4355$). Compared with beta-blocker users, adjusted relative risks (RRa) were 1.78 [95% CI 1.63–1.95] for antiepileptics and 1.03 [0.94–1.13] for neuroleptics. Relationships between switchback, patient demographic data and drug characteristics will be discussed.

Conclusion: A higher switchback risk to branded drugs was found among AEDs users compared to beta-blocker (+78%) or neuroleptic (+73%) users. These results could reflect a poor acceptance of switching AEDs to generic compounds in France.

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Keywords: generics, antiepileptic drugs, substitution.

CO-130

Generic substitution of antiepileptic drugs and loss of seizure control: a population-based case-crossover study

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Introduction: There are still controversies over pill substitution among antiepileptic drugs (AEDs): some studies claimed that switching between brand and generic AED (generic substitution) can lead to breakthrough seizures; other studies have refuted these concerns. We aimed at further estimating the association between generic substitution and loss of seizure control.

Patients and methods: We used data from the SNIIRAM database to identify a cohort of patients aged 18 years or more on January 2009 who filled a prescription in 2009–2011 for AED that had at least 1 brand-name and 1 generic form available on the French market (carbamazepine, lamotrigine, levetiracetam, topiramate or valproic acid). Patients with a medical history of cancer and women who gave birth (ICD-10 codes, O80-O84) were excluded, as patients receiving valproate registered as mood-stabilizer. We used a case-crossover design to assess the relationship between seizure-related hospitalization (SRH) and generic substitution (GS). Cases were identified as individuals with a SRH between January 2010 and December 2011 (ICD-10 codes, G40.x or G41.x). The index date was defined as the date of first occurrence in the inpatient file of G40-G41 pending a preceding hospitalization-free period of at least 1 year along with regular dispensations of targeted AED. The case period corresponded to the 3 months preceding the index date; the control period was defined as the 3 months immediately preceding the case period. GS was defined as a filled prescription for a generic AED that was preceded by a filled prescription for a brand-name counterpart. Matched odds ratio estimates (and 95% CIs) were based on the ratio of discordant pairs of GS events in case and control periods using conditional logistic regression model.

Results: The initial cohort included 679 387 adult patients, 66 315 with eligible SRH. Among them, 8379 were SRH free the year preceding the index date and received regular dispensations. Four hundred seventy-eight patients had a GS during the case period only, whereas 491 patients had a GS during the control period only; matched OR 0.97 [95% CI: 0.86, 1.10].

Conclusion: Generic substitution was not associated with an elevated risk of SRH.

Keywords: antiepileptic, generic substitution, seizure, case-crossover study, SNIIRAM database.