RESPIRATORY DISEASES

P1
MULTIVARIATE PATIENT SIMULATION FOR CLINICAL TRIAL OPTIMIZATION IN COPD
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Objectives: Clinical Trial Simulation (CTS) can be a valuable tool to improve drug development [1]. However, in order to obtain realistic simulation scenarios, the patients included in the CTS process must be representative of the target population. This is particularly important when covariate effects exist that may affect the outcome of a trial. The objective of this exercise is to evaluate the performance of different methods to simulate demographic covariates of patients for a Chronic Obstructive Pulmonary Disease (COPD) trial.

Methods: Virtual patients with varying demographic characteristics were simulated by re-sampling with replacement, sampling from a univariate distribution and sampling from a multivariate distribution. Simulations of continuous and categorical covariates were performed in R according to the method described by Tannenbaum et al. [2]. A KPD model was used to generate FEV1 responses in the COPD trials and results compared with the data from a real patient population.

Results: Covariate simulation using a multivariate distribution allows covariate correlations to be characterized using an empirical distribution. Moreover using the multivariate distribution is also possible to simulate new populations stratifying for specific covariates of interest.

Conclusions: Multivariate distribution methods may be applied to continuous and categorical covariates. This procedure is valuable for the optimization of the design of clinical studies in which covariate effects are known to influence treatment outcome (pharmacokinetics or pharmacodynamics).

References:

P2
THE COMBINED EFFECT OF DIAZEPAM AND VERAPAMIL ON TRACHEAL RESPONSE CAUSED BY ACETYLCHOLINE AND HISTAMINE IN GUINEA PIGS
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The goal of this in vitro study conducted upon experimental animals comprised assessment of the combined effect of diazepam and verapamil on the response of the smooth musculature of an isolated respiratory organ (trachea) of a guinea pig to histamine and acetylcholine. Thereby, local mechanisms of the smooth musculature were examined, and other neurohumoral influences were excluded. Guinea pigs of both genders, weighing between 500 and 700 g, were used in the experiment. The research involved a total of 10 guinea pigs that were euthanized, their tracheae being subsequently collected. The isolated organ samples were split into two groups (A and B). Acetylcholine, applied at concentrations between 1E-06 M and 1E-03 M, yielded a concentration-dependent smooth muscle contraction (slope: 24.98 ± 3.18, r = 0.99, P < 0.01); pD2 = 4.73; EC50 = 5.11 ± 0.91. A 1-min incubation of the isolated organ by a combination of diazepam and verapamil (1E-05M) significantly decreased the acetylcholine-induced contraction (slope: 22.1 ± 8.3; r = 0.99, P < 0.01); EC50 = 3.660 ± 0.19. Diazepam and verapamil brought about a substantial abatement of smooth muscle contraction in a concentration of 1E-04 M as well (slope: 14.31 ± 6.7, r = 0.93, P < 0.05); EC50 = 2.81 ± 0.782. In concentrations between 1E-06 M and 1E-03 M, histamine evoked a concentration-dependent contraction of the guinea pigs’ tracheal smooth muscle (slope: 25.38 ± 3.18, r = 0.96, P < 0.05) pD2 = 4.5, which was blocked by a concentration of 1E-05 M of the diazepam-verapamil combination.
TARGETED THERAPY IN ONCOLOGY

P3 CORRELATION BETWEEN DEVELOPMENT OF HYPERTENSION AND EFFICACY IN METASTATIC COLORECTAL CANCER PATIENTS TREATED WITH BEVACIZUMAB

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Introduction: Bevacizumab is a monoclonal antibody that targets vascular endothelial growth factor (VEGF) for treatment of metastatic colorectal cancer. Recently, much evidence has suggested that bevacizumab-induced hypertension might be predictive of the effect of bevacizumab. The aim of our study is to retrospectively assess the relationship between the onset of hypertension and the activity of bevacizumab in Japanese metastatic colorectal cancer patients.

Patients and Methods: Between July 2007 and December 2010, 36 patients (median age 66 years; 36–81 years) with metastatic colorectal cancer were assigned to receive bevacizumab in combination with either mFOLFOX6 (5-FU, levofofolute and oxaliplatin) or FOLFIRI (5-FU, levofofolute and irinotecan) at the Tokushima University Hospital. A patient who had increase by >20 mmHg in diastolic blood pressure or had increase to >150/100 or received antihypertensive treatment within four cycle after first infusion was defined as hypertensive.

Results: The objective response rate (ORR), disease control rate (DCR) and progression-free survival (PFS) were compared between the hypertensive group (n = 10) and non-hypertensive group (n = 26). ORR and disease control rate (DCR) were 60.0% and 100%, respectively, in the hypertensive group and ORR and DCR were 23.1% and 80.8%, respectively, in the non-hypertensive group. These differences were statistically significant (P < 0.05). The median PFS tended to be longer in the hypertensive group (65.0 weeks) than in the non-hypertensive group (40.0 weeks). Conclusion Our data suggested that bevacizumab-induced hypertension may be predictive of the effect of bevacizumab in Japanese metastatic colorectal cancer patients.

P4 VALIDATION OF A HPLC-MS/MS METHOD FOR THE DETERMINATION OF PLASMA DOCETAXEL CONCENTRATIONS

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Introduction: Docetaxel a member of the taxane drug family, along with paclitaxel (IS), is used in the treatment of cancer as a single agent or in combination therapy. It has a narrow therapeutic index and demonstrates inter-individual variability in pharmacokinetics. There is a strong correlation between drug concentrations and neutropenia. The aim of the study was to develop a HPLC-MS/MS method for the accurate determination of docetaxel in plasma.

Method: Docetaxel calibrator stock solutions were prepared in methanol to span the range 5–1000 ng/ml. Twenty-five micro litre of stock, 25 μl of IS (2 mg/ml) and 500 μl of water was added to 250 μl of plasma. A liquid–liquid extraction was performed with methyl-tert-butyl-ether. Samples were mixed on a horizontal shaker for 20 min at 70 rpm, and then centrifuged for 10 min at 2500 rpm. The aqueous layer was snap frozen and the organic layer transferred to clean 5 ml tubes and dried. Samples were reconstituted in mobile phase and 50 μl injected into the system. Samples were eluted using a Thermo Scientific Hypersil Gold C8 (50 × 2.2 mm, 5 μm) column using a gradient mobile phase consisting of 30% acetonitrile:water:1% formic acid (300 μl/min) for 1.5 min, 80% acetonitrile:water:1% formic acid (600 μl/min) for 0.5 min, then returning to the initial equilibration conditions (run time = 5 min). Peaks of interested eluted at 2.5 min. The HPLC consisted of an Agilent HP1000 system with detection via an API2000 triple quadrupole mass spectrometer using multiple reaction monitoring of docetaxel 808.1/226.2 m/z and paclitaxel 830/509 m/z. Turbo-ionspray was operated in positive ion mode. RESULTS Calibrators demonstrated within run CV% and biases% of <15% and <20% at the LOQ (5 ng/ml). Quality controls (25 and 250 ng/ml) demonstrated within run CV% of 16.9% and 12.7% and biases of 0.6% and -9.9%, respectively. The overall efficiency at 25 and 1000 ng/ml were 96.1% and 98.7%, respectively. The extract efficiency at these concentrations was 90.7% and 101.0%, respectively and matrix effects were -3.5% and +1.5%, respectively.

Conclusion: The method will be applied to the determination of plasma docetaxel concentrations in prostate and breast cancer patients.

P5 EFFECTS OF KETOCONAZOLE AND RIFAMPICIN ON THE PHARMACOKINETICS OF MIDOSTAURIN IN HEALTHY SUBJECTS

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Introduction: Midostaurin, a promising tyrosine kinase inhibitor (TKI) with known roles in hematopoiesis and leukemia, has been shown in vitro as a CYP3A4 substrate. Drug-drug interactions (DDIs) of midostaurin with CYP3A4 inhibitors (eg ketoconazole) and inducers (eg rifampicin) are expected.

Patients and Methods: Part 1: 36 subjects were randomized into two parallel groups, placebo or ketoconazole 400 mg QD for 10 days and a single 50 mg dose midostaurin on Day 6. Part II. Forty subjects were randomized into two groups, placebo or rifampicin 600 mg QD for 14 day and a midostaurin 50 mg single dose on day 9. PK parameters of midostaurin were determined with and without ketoconazole or rifampicin.

Results: In the presence of ketoconazole, the geometric means of Cmax and AUCinf of midostaurin decreased from 2892 to 1585 ng/ml and from 205911 to 19762 ng/h/ml, respectively, compared to placebo. The geometric mean T1/2 of midostaurin is prolonged from 23 to 5.1 hr in the presence of rifampicin. The geometric mean T1/2 of midostaurin decreased from 20.5 to 5.1 hr in the presence of rifampicin.

Conclusion: Inhibition of CYP3A4 by ketoconazole increases midostaurin exposure over 10-fold and induction of the enzyme by rifampicin decreases midostaurin exposure by over 90%, suggesting that midostaurin is a very sensitive CYP3A4 substrate (FDA Guideline (2006) ‘Drug Interaction Studies). The study was supported by Novartis Pharma AG.
P6 ESCITALOPRAM OXALATE, A SELECTIVE SEROTONIN REUPTAKE INHIBITOR, EXHIBITS CYTOTOXIC AND APOPTOTIC EFFECTS IN GLIOMA C6 CELLS
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Objective: Various antidepressants, mainly tricyclic antidepressants (TCAs) and selective serotonin reuptake inhibitors (SSRIs) have been reported to exhibit potent anticancer properties in different cancer cells. In this study, we evaluated the antiproliferative and apoptotic effects of escitalopram oxalate (25, 50, 100, 200 μM) on rat C6 glioma cells.

Methods: Cell proliferations were measured by MTT assay, apoptosis was observed by flow cytometric analysis on C6 cells.

Results: Significant decreases in the proliferation of C6 glioma cells were detected depending on increases in the escitalopram concentrations and incubation periods. When compared to controls, C6 cell proliferations after 24 h incubation were determined with 97.7%, 85.9%, 74.5% and 67.9% for 25, 50, 100 and 200 μM escitalopramoxalate respectively, while the cell proliferations after 48 h were established as 96.5%, 68.0%, 50.7% and 39.9% for 25, 50, 100 and 200 μM concentrations respectively. IC50 value of escitalopram was able to be calculated as 106.97 μM after 48 h. Based on annexin V-PI binding capacity for 25, 50, 100 and 200 μM escitalopram, apoptotic effects were determined as 17.0%, 22.3%, 12.5%, 7.8% respectively.

Conclusion: Based on our findings, escitalopram oxalate was observed to induce cytotoxic and apoptotic activities in C6 cells.

Key words: Escitalopram oxalate, C6 glioma cell line, NIH3T3 cell line, flow cytometry.

P7 REGULATION OF APOPTOTIC SIGNALING BY FLIP MODULATORY PROTEINS
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Introduction: Chemotherapeutic agents often stimulate apoptotic signalling inside cancerous cells, enabling their deletion during treatment. Chemotherapy-resistance arises from adaptation of apoptotic mechanisms, ensuring cancer cell survival. Here we report regulation of apoptotic signalling in human cancer cells by tumour necrosis factor (TNF)-induced signalling and FLIP (FLICE) (Fas-associated protein with death domain-like IL-1-converting enzyme-inhibitory protein). The long isoform, FLIP-L, regulates life and death to render resistance to death receptor-mediated apoptosis, either through intrinsic (mitochondrial) or extrinsic pathways. We tested the regulation by FLIP-L of the complex regulatory networks involved in cell survival/death responses in cancer.

Materials and Methods: Human embryonic kidney epithelial (HEK293) cells had FLIP-L levels reduced using lentiviral vectors stably expressing miRNA designed to knock-down FLIP-L protein expression (miRNA-FLIP-L KD). Neg-miRNA vector was expressed in control cells. Protein arrays were used to assess adaptation of key apoptotic signalling pathways.

Results: In miRNA-FLIP-L KD cells, mRNA levels were 40% of control levels, with FLIP-L protein expression being 35% of control levels. Silencing FLIP-L changed expression of proteins involved in intrinsic apoptosis, some cytoprotective protein adaptations, and several proteins regulating cell cycle progress. Also, FLIP-L-silenced cells have a lower rate of proliferation and cell cycle progression when compared to control cells, that difference being reduced after TNF treatment.

Conclusion: Overall, FLIP-L expression regulates rates of proliferation by changing only a few key regulatory proteins, mainly from the intrinsic TNF apoptotic pathways. These results suggest cancer cells evolved complex compensatory mechanisms to adapt to the absence of a key apoptotic regulatory proteins.

Acknowledgments: This study was supported by research funding in the form of grant support from the Association for International Cancer Research (AICR) and The Leukaemia and Lymphoma Research Foundation (LLRF).

P8 CURCUMIN SYNERGISTICALLY POTANTIATES APOPTOTIC AND CYTOTOXIC EFFECTS OF ATORVASTATIN ON HUMAN LUNG CANCER CELLS
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Introduction: Atorvastatin is widely used as an anticholesterol drug. However, the effect on cancer cells is still being investigated. Curcumin (diferuloylmethane), is a major naturally-occurring phenolic compound obtained from the rhizome of the plant Curcuma longa, which is used as a spice or yellow coloring agent for foods or drugs. Curcumin has exhibited several properties consistent with many potential cancer applications.

Materials and Methods: In this study apoptotic and cytotoxic effects of atorvastatin with the combination of curcumin on human lung cancer A549 cells were evaluated. Effects of curcumin and atorvastatin combination on cell morphology were determined by light (non-staining) and fluorescence (acridine orange/ethidium bromide staining) microscopy. Cell viability was measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The apoptotic effects were examined by AnnexinV-FITC/propidium iodide (PI) assay using flow cytometry, and apoptotic, necrotic and live cells were determined as percentage.

Results: While 12.5, 25, 50 μM atorvastatin provided an insignificant decrease in cell viability according to MTT assay after 24 and 48 h incubations, only 25 μM curcumin significantly reduced the cell viability. In combination treatments of 12.5, 25, 50 μM atorvastatin and 25 μM curcumin were determined significant decreases in cell viability at the rate of %33, %38 ve % 49 on A549 cells, respectively. While early and late apoptosis only with curcumin were 3.5% and 30.9% respectively, these rates with only atorvastatin were 3.4% and 7.2%. Also, in combination treatment of 50 μM atorvastatin and 25 μM curcumin it was determined that percentage of early apoptotic cells further increased to 12.5% on A549 cells by flow cytometric analysis.

Conclusion: In accordance with these results, it was observed that combination treatment of atorvastatin and curcumin was increased in both apoptotic and necrotic cells after 24 h incubation. Our results shows that 25 μM curcumin and 50 μM atorvastatin had a clear apoptotic-necrotic activity. Furthermore, we found that combination of atorvastatin and curcumin could be effective in the cancer-therapeutic treatment of human lung cancer.

Keywords: curcumin; atorvastatin; synergism; apoptosis, human lung cancer cell.
P9

AUTOPHAGY AND APOPTOSIS MODULATION IN OSTEOSARCOMA CELLS EXPOSED TO A NEW PALLADACYCLE COMPOUND

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Introduction: The goal of anticancer therapy is to compromise effectively tumor cells growth and survival, so as to cause cancer regression and prevent or, at least delay, its recurrence. As apoptosis is commonly inactivated in cancer, which is often associated with tumor progression rendering the cells resistant to chemotherapy and radiation-induced cell; the search for drugs able to modulate other events involved in cell death or survival is of great interest, such as the autophagic pathway.

Objectives: To evaluate the effectiveness of a compound called Biphosphinic Palladacycle Complex (BPC) against a human osteosarcoma cell line (SaOS-2) and determine the mechanism of BPC-induced cytotoxicity.

Methods: Cytotoxicity was determined by MTT reduction test. Using flow cytometry with PI and annexin-V-FITC simultaneously, we quantified necrosis and apoptosis, respectively. [Ca²⁺]c was measured in tumor cells loaded with fura-2AM and exposed to BPC. The cytosolic calcium origin was studied using FCCP, thapsigargin, Nigericin and Ca²⁺ free medium. Lysosomal membrane permeabilization and acidic vacuolar organelles (AVOs) were measured by confocal microscopy and flow cytometry, respectively using the metachromatic dye Acrindine Orange (AO). Involvement of Bax, a pro-apoptotic protein was also evaluated by GFP-Bax transfection assay.

Results: BPC induced SaOS-2 cells death with an IC₅₀ of 20 μM. This concentration induced 34.7% of apoptosis; 12.2% of necrosis and 12.9% of secondary apoptosis. Translocation of Bax from cytosol to mitochondria was also verified and it is probably contributing to the cell death induced by BPC. BPC also increased [Ca²⁺]c levels, which were derived from mitochondrial and lysosomal stores. After labeling cells with AO, flow cytometry detected a decrease of AVOs generation 12 h after BPC addition (20 μM). Confocal microscopy showed that BPC increased the lysosomal membrane permeability suggesting a possible role for lysosome acidic hydrolases in the cell death mechanism of the compound.

Conclusions: BPC-induced SaOS-2 cells death was accompanied by increased [Ca²⁺]c levels, which were mobilized from intracellular stores. This event was preceded by lysosomal membrane permeabilization, suggesting that this organelle is of a critical step for BPC induced cell death. The decrease number of AVOs formation also suggest that BPC is able to inhibit the final step of autophagy and that this event contributes to the effectiveness of BPC in eradicate SaOS-2 cells. Financial support: FAPESP, CNPq and CAPES.

P10


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Introduction: Metastatic Colorectal Cancer (mCRC) is one of the leading causes of death by cancer in Europe. In the 90’s, Irinotecan and Oxaliplatin treatments represent the traditional chemotherapeutic treat-
individuals with 3435TT (25.3%) genotype have an improved immune recovery and more pronounced decrease in viral load upon treatment. The CYP2D6 analysis revealed that 15 individuals (8.8%) are poor metabolizers. This is associated with high antiretroviral plasma concentrations, which results in decreased metabolic efficiency. Regarding the 5-FU response, the allele frequencies for DPYD*2A and del6bp (TYMS) are 0.9 and 32.6%, respectively. Currently, analysis of other functional variants in DPYD (T85C, T1679G, A2846T) and TYMS (1494del6bp) are ongoing, in order to provide a more accurate optimization of treatment in patients receiving 5-FU infusion therapy.

Conclusions: In summary, the results validate the clinical interest in pharmacogenetic analysis in patients treated with antiretrovirals and 5-FU. Furthermore, these data are relevant for the development of pharmacogenetic profiling in the Azores population. (Funded by the Azores Government: M121/I/002/2008 and M316/F/161/2009).

P12
THE EFFECT OF 3-ALKYL FENTANYL ANALOGUES ON ANTINOICEPTION AND BODY TEMPERATURE IN RATS: STRUCTURE-ACTIVITY RELATIONSHIP

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Introduction: It is well known that introduction of 3-methyl group in the piperidine ring of the fentanyl skeleton results in a significant increase in an analgesic activity. The aim of the present study is to evaluate analgesic activity and the body temperature of a newly synthesized fentanyl analogue, (+) cis-3-butyl fentanyl (B) and to compare it to the same activity of fentanyl (F) and (+) cis-3-methyl fentanyl (M) in rats.

Methods: Analgesic activity was assessed by tail-immersion test and the body temperature by insertion of a thermometer probe 5 cm in to the colon of unrestrained male Wistar rat.

Results: The relative order of analgesic potency was as follows: M (8) > F (1) > B (0.06). Equi-effective doses for analgesia (5xED50, 10xED50 and 20xED50) of B, M and F produced similar (P > 0.05; ANCOVA) hyperthermic responses with a mean deviation from baseline colonic temperature: 0.25 ± 0.08°C, 0.79 ± 0.10°C and 1.10 ± 0.07°C for B, 0.31 ± 0.07°C, 0.80 ± 0.08°C and 0.95 ± 0.15°C for M, and 0.22 ± 0.09°C, 0.98 ± 0.21°C and 1.19 ± 0.16°C for F. Also, the duration of both analgesic and hyperthermic activity of B is similar to F and significantly shortened in comparison to M (P < 0.05; ANCOVA).

Conclusion: It is concluded that introduction of 3-alkyl group larger than 3-methyl, reduce the potency in comparison to fentanyl, suggesting that the potency of 3-alkyl fentanyl analogues is significantly influenced by the voluminosity of the alkyl group. Also it is evident that introduction of 3-alkyl group in the piperidine ring of fentanyl skeleton affects analgesic and hyperthermic response in a similar way, indicating that both effects are mediated via the same receptor type, presumably μ receptor.
TRANSLATIONAL MEDICINE

P14 CLINICAL AND GENETIC RISK FACTORS FOR SEVERE ANEMIA IN KIDNEY ALLOGRAFT RECIPIENTS WITH HISPANIC ETHNICITY

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Introduction: Anemia is one of the common complications after kidney transplantation. Post-transplantation anemia occurs both early and then later after transplantation and is strongly associated with significant cardiovascular morbidity and hospitalization. The objective of this study was to identify clinical and genetic risk factors associated with severe anemia in Hispanic renal transplant patients.

Methods: A total of 430 Hispanic transplant patients who had received kidney at St. Vincent Medical Center were included in this study. Severe anemia was defined as hemoglobin (Hgb) <10 g/dl which required treatment. Data regarding ‘early anemia’ patients were collected at 0, 1, 3, 6 months after transplantation, while data regarding ‘late anemia’ patients were collected at 1, 3, 4, 5 years after transplantation. Factors analyzed were erythropoiesis-stimulating agent, ACEi/ARB, anti-viral and anti-bacterial agents, immunosuppressants, SCr, eGFR, albumin, and Hgb. The genotypes of the angiotensin 1 receptor (AT1R), angiotensin (AGT) and angiotensin converting enzyme (ACE) were also obtained. Statistical analysis was performed with SPSS (version 18) software. A multiple logistic regression model using post-transplant data was created to control the effects for other predictors.

Results: In early severe anemia, gender (female vs. male, OR = 3.1 P = 0.001), number of transplants (transplants >= 2 vs. 1, OR = 4.6, P = 0.001), AT1R genotype (rs5182, CC vs. AA+AC, OR = 1.9, P = 0.043), donor type (deceased vs. living, OR = 2.1, P = 0.038), Scr in 6th month (Scr>1.5 vs. Scr≤1.5, OR = 4.1, P < 0.001), sirolimus (sirolimus vs. non-sirolimus users, OR = 2.1, P = 0.039) remained significant after logistic regression. In late severe anemia, donor type (deceased vs. living, OR = 7.9, P = 0.001), AGT genotype (rs5051, TT vs. CT+CC, OR = 2.3 P = 0.044), serum creatinine (Scr>1.5 vs. Scr≤1.5, OR = 6.0, P < 0.001) remained significant after multistep binary logistic regression. All other factors were not statistically significant.

Conclusion: Our data suggest that different clinical and genetic risk factors may contribute to early severe anemia compared with late severe anemia in Hispanic kidney transplant recipients. However, the angiotensin pathway is important in both early (AT1R) and late (AGT) severe anemia.

P15 DISCRIMINATION OF A1555G AND C1494T POINT MUTATIONS IN THE MITOCHONDRIAL 12S RNA NONSYNDROMIC DEAFNESS BY ON/OFF SWITCH
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Introduction: To apply the on/off switch of 3’ exo-nuclease in single base discrimination of A1555G and C1494T mutations in the highly conserved site of the mitochondrial 12S rRNA which are the hot spot mutations associated with both aminoglycoside-induced and nonsyndromic deafness in many families.

Methods: Allelic specific primers targeting wild type and mutation type templates were designed with 3’ terminal phosphorothioate modification. A large amount of templates of wild type and mutation type were prepared using cloned plasmids containing relevant DNA fragments respectively. Two-directional primer extension was performed using polymerases with and without 3’ exonuclease activity.

Results: Whether Single or multiplex PCR, amplified by exo+ polymerase allelic specific primers targeting wild type allele were extended while no products were generated from primers targeting point-mutated deafness-related allele, and also allelic specific primers targeting point-mutated deafness-related mutation type allele were extended while no products were generated from primers targeting wild type allele. As a control, exo- polymerase yielded products from both types of primers.

Conclusion: These data suggest that the off-switch mediated by exo- polymerase is more reliable as compared to exo+ polymerase in the diagnosis of monogenic diseases and the novel ‘on/off’ switch has enormous application in systematic and extended mutational screening of the 12S rRNA A1555G and C1494T mutations not only for hearing loss patients but also for normal subjects before the use of aminoglycoside antibiotics. The project was supported by the Chinese National 863 major grant (2008AA02Z436).

P16 MICROGlia- THE NEW PARADIGM IN THE TREATMENT OF BRAIN DISEASES?
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Neurodegenerative diseases present challenges in it’s diagnosis and treatment, this contributes to poor outcomes in it’s treatment. Treatment paradigms have been palliative and generally from “the outside in". The study of microglial cells has become a key to the understanding of the mechanisms underlying neuropathologies. Microglia not only form the first line of defence but also serve to control the immune response of the brain. They are sensitive and react immediately to changes in the brain environment, in an attempt to maintain homeostasis. On their activation the blood brain barrier becomes less impenetrable thus forming an essential link between the CNS, and the peripheral immune system, (1)

This responsiveness of microglia activation has been detected prior to any neurodegeneration and may be neuroprotective or neurotoxic depending on the degree of activation of the microglia cells. Monitoring microglial activation would give an indication of disease progression and an indication of when to begin therapy. This better understanding of disease progression could lead to individually tailored regimen based on the patient responsiveness to therapy. Are there biomarkers of microglial activation that can be used therapeutically to achieve this end and can the neuroprotective aspects of microglial cells be harnessed therapeutically in delaying disease progression . The role of microglia in a selection of disease states is presented . The challenges and opportunities presented by this proposed new paradigm of treatment is presented and is discussed.

Reference:
P18
GENERATION OF AN OATP1B1-UGT1A1-MRP2 TRIPLE-TRANSFECTED MDCK CELL LINE: IDENTIFICATION OF EZETIMIBE GLUCURONIDE AS SUBSTRATE OF MRP2

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Introduction: Ezetimibe, a cholesterol lowering drug undergoing extensive entero-intestinal and entero-hepatic circulation, and its phenolic glucuronide were previously shown to interact with OATP1B1. The hepatic uptake transporter OATP1B1 (gene symbol SLCO1B1) is localized in the basolateral membrane of human hepatocytes and mediates the uptake of substances from blood. Furthermore, the intracellular glucuronidation of ezetimibe is mediated by the uridine diphosphate-glucuronosyltransferase 1A1 (UGT1A1). Nevertheless, the molecular mechanism of the final hepatic excretion step of ezetimibe glucuronide into bile still remains unknown.

Methods: The metabolism and subsequent excretion of ezetimibe were investigated in time-dependent vectorial transport experiments using a newly established triple-transfected MDCK cell line stably expressing the uptake transporter OATP1B1, the phase-2-enzyme UGT1A1 and the export pump MRP2. Several single- and double-transfected cell lines served as controls in all experiments. Ezetimibe glucuronide was determined by LC/MS/MS.

Results: The simultaneous expression of the transporters OATP1B1 and MRP2 together with the phase-2-metabolizing enzyme UGT1A1 was verified by mRNA expression and immunoblot analyses. Vectorial transport experiments with monolayers of control cells and the triple-transfected cell line using unlabeled ezetimibe revealed that ezetimibe glucuronide accumulated to a significantly higher amount in the apical compartment in the triple-transfected cell line compared to the OATP1B1-UGT1A1 double-transfected cell line (vectorial transport ratio: 7.2-fold). No ezetimibe glucuronide was detectable in the apical compartment when the other control cell lines were used.

Conclusion: Using a newly established triple-transfected OATP1B1-UGT1A1-MRP2-MDCK cell line, we demonstrated that MRP2 is involved in the transport of ezetimibe glucuronide across the hepatocyte canalicular membrane into bile. This work was supported by a grant of the Doktor Robert Pfleger-Stiftung.

P19
CANNABIDIOL CHANGES THE EXPRESSION PROFILE OF MDR PROTEINS IN THE HUMAN PLACENTAL BARRIER

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Introduction: Cannabis is the most commonly used illegal drug among pregnant women. Cannabidiol (CBD) is a major non-psychotropic cannabinoid found in Cannabis sativa. BCRP and P-gp are efflux transporters expressed at the apical membrane of the syncytiotrophoblast in the human placental barrier. To day, very little is known about the influence of cannabinoids on BCRP and P-gp in the human placenta.

Methods: The focus of our study was CBD effect on the expression of BCRP and P-gp in MCF7 cells, in a comparable trial. We showed that chronic exposure to CBD may alter P-gp and BCRP expression in the human placenta but, CBD effects seem to be cell type specific. The elevation seen in BCRP levels could be compensatory to the reduction of P-gp protein levels.

Conclusions: These findings indicate that Cannabis consumption during pregnancy (especially throughout the first trimester) could jeopardize fetal wellbeing by changing the expression profile of active efflux placental barrier transporters.

P20
ALTERED EXPRESSION OF TRANSPORTERS AND DRUG-METABOLIZING ENZYMES IN THE MICE WITH NEUROPATHIC PAIN

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Introduction: It is known that morphine does not produce its full analgesic effect on neuropathic pain which is common in cancer patients. The reason for this ineffectiveness is considered to be a decrease in opioid µ-receptor function in neuropathic pain. In this study, pharmacokinetic analysis was performed to examine the mechanisms of this morphine resistance in neuropathic pain.

Material and Methods: The model mice with neuropathic pain were prepared using the method reported by Seltzer et al. The expression level and activity of transporters and drug metabolizing enzymes that affect the pharmacokinetics of morphine were examined using PCR, Western blot, HPLC, and Immunohistochemistry.

Results: In the model mice with neuropathic pain, increases in the expression level of P-gp in the intestines, expression level and activity of Ugt in the liver, and expression level of P-gp in the blood-brain barrier were observed. An increase in the expression level of MRP2 and decrease in MRP3 in the liver were also observed.

Conclusion: The mechanisms of morphine resistance to neuropathic pain were postulated as follows: (i) The amount of morphine absorbed into the body decreases with the increase of P-gp expression level in the intestines. (ii) The total amount of morphine decreases because of increased metabolism of morphine attributable to the increase of hepatic Ugt activity. (iii) The analgesic activity of morphine decreases due to a decreased concentration of morphine in the brain attributable to the increase in the P-gp expression level at the blood-brain barrier.
USE OF BIOMARKERS IN CLINICAL TRIALS

P22
INFLUENCE OF GLUTATHIONE S-TRANSFERASE M1, T1, A1, P1 POLYMORPHISMS ON ORAL BUSULFAN PHARMACOKINETICS IN ADULTS SUFFERING FROM MYELOPROLIFERATIVE DISORDERS UNDERGOING HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Introduction: Busulfan (BU) is often used in high dose for myeloablation before hematopoietic stem cell transplantation (HSCT). BU has been implicated in certain hematopoietic stem cell transplantation toxicities. Gene polymorphisms in specific members of the glutathione S-transferase (GST) gene family (M1, T1, A1, P1) involved in BU metabolism, may play a role in the wide inter-patient variability in systemic BU concentrations such as in children undergoing HSCT for the treatment of congenital hemoglobinopathies.

Patients and Methods: This prospective pharmacokinetic/pharmacogenetic (PK/PGx) study provides an integration of clinical data regarding the BU pharmacokinetics and GSTM1, GSTT1, GSTA1 and GSTP1 genotypes of 34 adults who suffered from different myeloproliferative disorders. The myeloablative conditioning regimen was based on high-dose oral BU (1 mg/kg every 6 h for 4 days) followed by intravenous cyclophosphamide.

Pre-transplant peripheral blood samples were collected and genomic DNA was extracted using Qiamp DNA mini kit (Qiagen, Germany). Plasma BU concentrations were measured by gas chromatography with an electron capture detector (Hewlett Packard Corporation, USA). The lower limit of detection was 0.2 μM. A non-compartmental analysis (NCA) model for extra-vascular absorption for oral delivery was applied (Kinetica 4.4- MA, USA) for BU pharmacokinetic analysis.

Results: The data demonstrate an association of GSTP1 heterozygote genotype and oral BU area under the concentration-time curve (AUC) (P = 0.04) and a relative association with oral BU clearance-F/kg body weight (P = 0.07). In addition, association between time to engraftment (days) was depicted with the GSTT1 genotype (25 subjects were wild type and eight subject were null) (P = 0.03). Graft vs. host disease (GVHD) (GVHD) was partially associated to the GSTA1 homozygote genotyping (P = 0.08). No other associations had been observed between the pharmacokinetic parameters of oral BU and the GST genotyping in those subjects.

Conclusions: GSTP1, GSTT1 and GSTA1 genotyping prior to HSCT in adults suffering from myeloproliferative disorders may allow better prediction of oral BU-AUC and the BU oral clearance with the need for BU dose adjustment, as well as prediction of transplant related outcomes. This study was funded in part by the Israel Cancer Association Grant and by the Ministry of Health Chief Scientific Officer Grant.

P23
CYP2C9 PHENOTYPING USING A SINGLE-POINT DRIED BLOOD SPOT AFTER ORAL ADMINISTRATION OF FLURBIPROFEN

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Background: Flurbiprofen (FLB) is widely accepted as a reliable probe for CYP2C9 activity assessment. However, only urinary measures of metabolism have been validated using an over-night or 8 h urine collection which is tedious, time consuming and uncomfortable for the patients and for the medical staff. In this study, we investigated whether a single blood measurement using a new and minimally invasive technique (dried blood spot) consisting of a 5 ul blood sampling using a small finger prick is suitable for CYP2C9 phenotype.

Methods: Ten healthy volunteers genotyped for the CYP2C9 received flurbiprofen (50 mg) alone in session 1, flurbiprofen and the CYP2C9 inhibitor flucloxacillin (400 mg) and in session 2 and in session 3 they were pretreated for 4 days by rifampicin (600 mg) and at day 5 they received flurbiprofen (50 mg) with the last dose of rifampicin. Plasma and DBS samples were obtained at 0, 0.5, 1, 2, 4, 6 and 8 h after drug administration and urine was collected from 0 to 8 h. FLB and OH-FLB were determined in DBS, plasma and urine using validated analytical methods.

Results: FLB and OH-FLB pharmacokinetic parameters were comparable between DBS and plasma. As expected, FLB and OH-FLB plasma concentration was twofold higher than DBS which is related to the hematocrit. Statistically significant differences in the OH-FLB/FLB metabolic ratios in urine (8 h), plasma (2 h) and DBS (2 h) were observed between the three sessions. Good correlations were obtained between urine, plasma and DBS metabolic ratios.

Conclusion: Single-point method using a DBS provides a reliable and a minimally invasive method of predicting CYP2C9 activity.

P24
CONTRIBUTION OF CYP2C8 AND CYP3A4 TO THE MAIN METABOLIC PATHWAY OF IMATINIB IN VITRO

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The tyrosine kinase inhibitor imatinib is predominantly eliminated as metabolites that are excreted via the bile. According to imatinib product information, the main enzyme involved in its metabolism is cytochrome P450 (CYP) 3A4. However, in a recent in vitro study, the formation of the main metabolite N-desmethylimatinib was also catalysed by CYP2C8. Using pooled human liver microsomes (HLM) and human recombinant CYP isoforms together with low, clinically relevant substrate concentrations (0.1–1 μM), we re-examined the contributions of different CYP enzymes, particularly those of CYP2C8 and CYP3A4, to the main metabolic pathway of imatinib and the further metabolism of N-desethylimatinib. In HLM, CYP3A4 inhibitors (ketocazone and troleandomycin) inhibited it by approximately 35%. However, CYP2C8 and CYP3A4 inhibitors inhibited imatinib N-demethylation to a similar degree (by 50–70%). Similarly, recombinant CYP2C8 catalysed imatinib N-demethylation with the same intrinsic clearance as did CYP3A4. For CYP2C8, the intrinsic clearance calculated from N-demethylation kinetics was almost identical to that based on depletion of 0.1 μM imatinib, indicating that N-demethylation accounts for the majority of imatinib metabolism by CYP2C8. By contrast, N-demethylation seemed to account for only one third of imatinib metabolism by CYP3A4. Moreover, the further biotransformation of N-desethylimatinib was mainly mediated by CYP3A4, with a small contribution by CYP2C8. In conclusion, CYP2C8 and CYP3A4 are the main enzymes mediating the formation and further metabolism of the active metabolite of imatinib at clinically relevant substrate concentrations in vitro.
P25
POLYMORPHISMS IN THE CYP3A4, CYP3A5 AND ABCB1 GENES MODIFY TACROLIMUS PHARMACOKINETICS AND PHARMACODYNAMICS IN RENAL TRANSPLANT
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Introduction: The calcineurin inhibitor tacrolimus is an immunosuppressant drug widely used for the prevention of organ rejection following transplantation. This agent is a metabolic substrate for CYP3A enzymes and is transported out of cells via the ABCB1-encoded P-glycoprotein. We examined the association of common single nucleotide polymorphisms (SNPs) in CYP2J2 and CYP2C8, which are genes involved in kidney homeostasis by producing epoxyeicosatrienoic acids. We studied the occurrence of SNPs in CYP2J2 and CYP2C8, which are genes involved in kidney homeostasis by producing eicosapentaenoic acids.

Patients and Methods: The existence of the CYP3A4*1B, CYP3A5*3, ABCB1 3435C>T, 2677G>T/A and 1266C>T, CYP2J2*7 and CYP2C8*3 SNPs was assessed by PCR-RFLP methods and direct sequencing in 103 renal allograft recipients. Doses and corrected concentrations of tacrolimus were recorded at first week, 1 month, 5 months and 1 year after transplant. Likewise, the occurrence of acute graft rejection, and toxicity (nephrotoxicity, neurotoxicity and gingival hyperplasia) was also examined.

Results: CYP3A4*5 expressors had lower tacrolimus concentration/dose ratios (ng/ml/mg) than non-expressors (CYP3A5*3/*3 carriers) at first week (60.9 ± 37.1 vs. 113.9 ± 75.9; P = 0.006), 1 month (63.4 ± 30.6 vs 126.7 ± 58.8; P = 0.002), 5 months (88.9 ± 56.8 vs 166.1 ± 75.3; P = 0.004) and 1 year (81.5 ± 56.3 vs 189.8 ± 107.1; P < 0.001) after transplant. Likewise, patients carrying the CYP3A4*1B variant showed lower ratios than CYP3A4*1A/*1A carriers at the same time points (110 ± 75.7 vs. 54.3 ± 24.5, P = 0.020; 120.5 ± 59.6 vs. 59.1 ± 24, P = 0.025; 160.5 ± 76.2 vs. 62.4 ± 41, P = 0.005 and 178.6 ± 106.7 vs 57 ± 17.3, P = 0.001). Moreover, carriers of the CYP3A5*1 or CYP3A4*1B alleles showed higher dose requirements at different time points post transplant (data not shown).

Conclusion: Our results suggest that CYP3A SNPs are associated with tacrolimus pharmacokinetics, whilst the ABCB1 haplotype may play a role in the drug-induced toxicity in renal transplant recipients.

P26
CYP2C9*3 ALLELE MARKEDLY AFFECTS THE PHARMACOKINETICS AND PHARMACODYNAMICS OF MELOXICAM
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Introduction: Meloxicam is an active metabolite of meloxicam, a selective histamine H1-receptor antagonist and is used for the treatment of seasonal allergic rhinitis and chronic idiopathic urticaria. It is reported that the biliary excretion of fexofenadine is mainly mediated by Mrp2 in mice. C-24T single nucleotide polymorphism (SNP) in ABCC2, gene that is encoding Mrp2 efflux transporter, is known to decrease the efflux activity and function. We investigated the effects of ABCC2 C-24T polymorphism on the pharmacokinetics of fexofenadine in healthy Korean volunteers.

Methods: Twelve subjects were selected and they were divided into two groups according to ABCC2 C-24T genotype, ABCC2 -24CC (CC, n = 8) and ABCC2 -24TT (TT, n = 4). Each subject received a single oral dose of 180 mg fexofenadine, and plasma concentration of fexofenadine were monitored by HPLC in plasma samples collected up to 36 h after drug intake.

Results: In CYP2C9*1/*3 subjects, half-life longer (P < 0.001), oral clearance lower (P < 0.01) and area under the plasma concentration-time curve (AUC) higher (P < 0.001) than those in homozygous CYP2C9*1 subjects. The AUC (8.0-fold and 4.9-fold, respectively) and half-life (5.1-fold and 3.1-fold, respectively) in CYP2C9*3/*3 was higher than those in CYP2C9*1/*1 and CYP2C9*1/*3 groups. The rate of TXB2 production was significantly lower in the CYP2C9*1/*3 and *3/*3 genotypes than in the CYP2C9*1/*1 genotype.

Conclusion: The CYP2C9*3 alleles seems to be associated with decreased metabolism and increased pharmacodynamic effects of meloxicam.

P27
EFFECTS OF CYP2C9*13 ALLELE ON THE PHARMACOKINETICS OF IRBESARTAN
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Introduction: Irbesartan is metabolized by polymorphic CYP2C9 to inactive metabolite. CYP2C9*13 allele show impaired activity towards a number of substrates both in vitro and in vivo. Unlike CYP2C9*3, which has been extensively studied in humans, clinical studies of CYP2C9*13 have been limited by the difficulty in finding subjects carrying this low-frequency allele. To evaluate the effect of CYP2C9*13 allele on the pharmacokinetics of irbesartan in healthy volunteers.

Methods: A 150 mg oral dose of irbesartan was given to 18 Korean volunteers with different CYP2C9 genotypes (12 and 6 carriers of CYP2C9*1/*1 and *1/*3 genotypes, respectively). Irbesartan was analyzed by HPLC in plasma samples collected up to 36 h after drug intake.

Results: In CYP2C9*1/*3 subjects, the maximum plasma concentration (Cmax) of irbesartan significantly higher (P < 0.01), half-life longer (P < 0.001), oral clearance lower (P = 0.002) and area under the plasma concentration-time curve (AUC) higher (P < 0.001) than those in homozygous CYP2C9*1 subjects.

Conclusion: The CYP2C9*13 alleles seems to be associated with decreased metabolism of irbesartan.

P28
ASSOCIATION BETWEEN ABCC2 -24C>T POLYMORPHISM AND THE PHARMACOKINETICS OF FEXOFENADINE
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Introduction: Fexofenadine, an active metabolite of terfenadine, is a selective histamine H1-receptor antagonist and is used for the treatment of seasonal allergic rhinitis and chronic idiopathic urticaria. It is reported that the biliary excretion of fexofenadine is mainly mediated by Mrp2 in mice. C-24T single nucleotide polymorphism (SNP) in ABCC2, gene that is encoding Mrp2 efflux transporter, is known to decrease the efflux activity and function. We investigated the effects of ABCC2 C-24T polymorphism on the pharmacokinetics of fexofenadine in healthy Korean volunteers.

Methods: A 150 mg oral dose of fexofenadine was given to 18 Korean volunteers with different ABCC2 genotypes (11, 8 and 2 carriers of ABCC2 -24CC, -24TC and -24TT genotypes, respectively). Fexofenadine was analyzed by HPLC in plasma samples collected up to 36 h after drug intake.

Results: In ABCC2 -24CC subjects, half-life longer (P < 0.001), area under the plasma concentration-time curve (AUC) higher (P < 0.001) than those in homozygous ABCC2 -24TT subjects. The AUC (8.0-fold and 4.9-fold, respectively) and half-life (5.1-fold and 3.1-fold, respectively) in ABCC2 -24CT was higher than those in ABCC2 -24CC and ABCC2 -24TT groups. The rate of TXB2 production was significantly lower in the ABCC2 -24CC and -24CT genotypes than in the ABCC2 -24TT genotype.

Conclusion: ABCC2 -24CT polymorphism is not likely to influence the pharmacokinetics of fexofenadine.
P29
EFFECTS OF CYP2D6*10/*10 GENOTYPE ON THE PHARMACOKINETICS OF ATOMOXETINE AND ITS METABOLITES

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Introduction: Atomoxetine (AT) is a nonstimulant indicated for the treatment of attention deficit hyperactivity disorder. 4-Hydroxyatomoxetine (4-HAT), a pharmacologically active metabolite, is primarily formed by CYP2D6. N-demethylatomoxetine (N-DMAT) is formed by CYP2C19, but has substantially less pharmacological activity compared with AT. We evaluated the effect of CYP2D6*10/*10 genotype on the pharmacokinetics of AT and its metabolites.

Methods: A 40 mg oral dose of AT was given to 22 Korean volunteers with different CYP2D6 genotypes (12 and 10 carriers of CYP2D6*wt/*wt and *10/*10 genotypes, respectively). Plasma AT, 4-HAT, and N-DMAT were analyzed by LC-MS/MS in plasma samples collected up to 24 h after drug intake.

Results: The active moiety of AT in CYP2D6*10/*10 was 200% higher than that in CYP2D6*wt/*wt (P < 0.001). In addition to, AUC of N-DMA in CYP2D6*10/*10 was 380% higher than that in CYP2D6*wt/*wt (P < 0.001). Compare with CYP2D6*wt/*wt, CYP2D6*10/*10 have a lower AUC ratio of 4-OHAT over AT (P < 0.001) and a higher AUC ratio of N-DMAT over AT (P = 0.008).

Conclusions: The mean exposure to active moiety of AT is markedly higher in subjects with the CYP2D6*10/*10 genotype than in those with the CYP2D6*wt/*wt genotype. These results indicate that further evaluation of the clinical significance of the CYP2D6*10 allele in the dosing of atomoxetine is needed.

P30
PANTETHINE RAISES PLASMA LEVELS OF CALCITONIN, GENE-RELATED PEPTIDE AND VASOACTIVE INTESTINAL POLYPEPTIDE IN HEALTHY VOLUNTEERS

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Introduction: Pantethine is known to increase gastrointestinal motility and improve ileal function. To determine the pharmacological effect of pantethine on levels of gastrointestinal peptide, we examined the effects of pantethine on plasma levels of calcitonin gene-related peptide (CGRP)-, vasoactive intestinal polypeptide (VIP)-, motilin- and substance P (SP)-like immunoreactive substances (IS) in healthy subjects.

Methods: An open-labeled crossover study was conducted on five healthy volunteers, which was approved by the Ethics Committee of Oita University. Each subject was administered a single oral dose each of pantethine and placebo at intervals of 1 month. Venous blood samples were collected before and 20–240 min after each administration. Plasma levels of CGRP, VIP, motilin and SP-IS were measured using a highly sensitive enzyme immunoassay.

Results: A single oral dose of pantethine resulted in significant increases in plasma CGRP-IS level at 40, 60 and 120 min compared with the levels after placebo administration. Plasma VIP-IS level was also significantly higher at 40 and 60 min after pantethine administration compared with placebo. On the other hand, no significant changes in plasma motilin- and SP-IS level were observed after the administration of pantethine.

Conclusion: Our results suggest that the pharmacological effects of pantethine may be closely related to changes in plasma CGRP- and VIP-IS levels, which are related to regulation of gastrointestinal function.

P31
COMPARATIVE EFFECTS OF IRBESARTAN AND AMLODIPINE ON OXIDATIVE STRESS IN HEALTHY ADULTS

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Introduction: Oxidative stress plays a major role in the development of cardiovascular diseases. It is important to know whether cardioiology drugs often used in clinical practice influence oxidative/antioxidative balance. 8-Isoprostanoglandin 2α (8-iso-PGF2α) and hydroxynonenal (HNE) have been shown to be useful for the assessment of oxidative stress in vivo. The aim of the study was to compare the effects of irbesartan and amlodipine on oxidative stress in healthy adults.

Methods: This was a randomized, open-label, 3-way crossover study in 12 healthy men. All subjects received the following treatments orally once daily for 7 days: irbesartan 150 mg plus amlodipine 5 mg (group A), irbesartan 150 mg (group B), or amlodipine 5 mg (group C). Oxidative stress status was determined by 8-iso-PGF2α and HNE-His adduct concentrations on day 1 (baseline) and day 8.

Results: A significant decrease in 8-iso-PGF2α and HNE-His adduct concentrations was observed on day 8 compared with day 1 (9.49 ± 40.1 vs. 64.6 ± 13.6 pg/ml and 3.19 ± 0.82 vs. 2.81 ± 0.68 µg/ml, respectively, P < 0.05) in the group A. However, no significant change in 8-iso-PGF2α and HNE-His adduct concentrations was observed in the group B (P > 0.05). There was a significant change in HNE-His adduct concentrations (3.52 ± 0.96 vs. 3.11 ± 0.74 µg/ml, P < 0.05) and no significant difference for 8-iso-PGF2α between day 8 and day 1 in the group C (P > 0.05).

Conclusion: Irbesartan and amlodipine combination was more effective for inhibition of oxidative stress than administration of irbesartan or amlodipine alone in the healthy adults.

P32
CARRIAGE OF CYP2C9*3 ALLELE IS ASSOCIATED WITH MORE RAPID DEVELOPMENT OF EROSIIVE AND ULCERATIVE LESIONS OF STOMACH AND DUODENUM IN LONG-TERM USE OF NONSTEROIDAL ANTIINFLAMMATORY DRUGS IN RUSSIAN PATIENTS WITH OSTEARTHRITIS

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Introduction: Nonsteroidal antiinflammatory drugs (NSAIDs) are most often used for a long time in patients with osteoarthrosis to relieve pain. However, the development of erosions and ulcers in the stomach and duodenum is the main problem in prolonged use of NSAIDs. Search for genetic predictors of this complication will allow a personalized approach to the choice of both the NSAIDs themselves and their doses, which should improve the safety of treatment. In this case, one of the candidate genes is the gene CYP2C9, encoding key enzymes of biotransformation of many NSAIDs, and, in particular, the diclofenac.

Objective: To evaluate the association between carriage of allele CYP2C9*3 and the early development of erosive and ulcerative lesions of the stomach and duodenum in long-term use of NSAIDs in Russian patients with osteoarthrosis.

Materials and Methods: The research included 98 patients with osteoarthrosis (68 women and 30 men) aged 59 ± 10.3, which were using diclofenac. In all patients the development of erosions or ulcers of the stomach or duodenum (which were regarded as manifestations of NSAID gastropathy) was confirmed according to the results of esophago-gastro-duodenoscopy. All patients underwent genotyping for CYP2C9 (identification of alleles CYP2C9*2 and CYP2C9*3) by polymerase chain reaction (PCR) after preliminary DNA extraction from leukocytes of...
periphery blood. The significance of differences was assessed using the Fisher criterion.

**Results:** According to the results of genotyping, 18 patients were carriers of CYP2C9*3 (genotypes CYP2C9*1/*3, CYP2C9*3/*3 and CYP2C9*2/*3) and 80 patients were not carrying the allele CYP2C9*3 (genotypes CYP2C9*1/*1, CYP2C9*1/*2, CYP2C9*2/*2). Of 18 patients carrying the allele CYP2C9*3, the erosive and ulcerative lesions developed during the first 3 months of NSAID in 10 patients (55.6%), of 80 patients who were not carrying the allele CYP2C9*3, the erosive and ulcerative lesions developed in the first 3 months in 22 patients (27.5%). The erosive and ulcerative lesions in the first 3 months of NSAID use significantly more often developed in carriers of CYP2C9*3 allele compared with patients not carrying the CYP2C9*3: 55.6% vs 27.5%, P = 0.0283, OR = 2.02 (ID95% 1.71–3.85).

**Conclusion:** For Russian patients with osteoarthritis carriage of allele CYP2C9*3 is associated with the development of erosive and ulcerative lesions of the stomach and duodenum in the first 3 months of NSAID use.

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**P33**

### THE INFLUENCE OF IRBESARTAN ON CIRCULATING MICRONAS IN HEALTHY CHINESE VOLUNTEERS


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**Introduction:** MicroRNAs (miRNAs), 21 to 25 nucleotide, endogenous non-coding RNAs, have been identified as a fine-tuner in numerous cellular processes, including proliferation, differentiation, apoptosis, and metabolism. Recently, studies demonstrated that miRNAs could be detected in plasma and serum, and the levels of some circulating miRNAs has been reported to vary significantly in some pathological conditions, such as a variety of cancers, tissue damage and drug treatment. As with all angiotensin II receptor antagonists, irbesartan is indicated for the treatment of hypertension. In this study, we investigated the influence of irbesartan on miRNAs expression levels in plasma.

**Methods:** Total RNAs were extracted from plasma samples of eight healthy Chinese volunteers collected before and after multiple-dosage oral administration of irbesartan for 7 days. The differentially expressed miRNAs selected by TaqMan Low Density Arrays screening and miRNA-network analysis were validated by Real-Time PCR in another eight volunteers administered likewise.

**Results:** Fourteen differentially expressed miRNAs were selected by miRNAs profile. The validation results showed that miRNA-25 was up-regulated to 2.4-fold changes and miRNA-323-3p was down-regulated to 2.0-fold changes after administration, and wilcoxon test showed that the differences were significant. Then, Bivariate correlation analysis exhibited that the down regulation of miRNA-323-3p correlated with the change of blood pressure by irbesartan but none with drug exposure.

**Conclusion:** Multiple-dosage oral administration of irbesartan could modulate circulating miRNAs expression in healthy Chinese. This work was supported by the National Science and Technology Major Project (No.2008ZX09312-018)

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**P34**

### CYP2C9 GENOTYPE DOES NOT AFFECT THE RESPONSE TO WARFARIN DURING THE FIRST 6 DAYS IN CHINESE PATIENTS

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**Background:** Genetic variants of the enzyme that metabolizes warfarin, cytochrome P-450 2C9 (CYP2C9), and of a key pharmacologic target of warfarin, vitamin K epoxide reductase (VKORC1), contribute to differences in patients’ responses to various warfarin maintenance doses, but the role of CYP2C9 variants during initial anticoagulation is not clear.

**Methods:** In 293 Chinese valve replacement patients who starting warfarin therapy, we assessed CYP2C9 genotypes (CYP2C9 *1/*2, *1/*3, *2/*2, *3/*3), clinical characteristics, response to therapy (as determined by the international normalized ratio [INR]), and bleeding events. The study outcomes were the time to the first INR within the therapeutic range, the total warfarin dose requirement in 6 days, and INR within the therapeutic range in the day 6.

**Results:** As compared with patients with the *1/*1 genotype, patients with the *2 or *3 genotype of CYP2C9 had a same time to the first INR within the therapeutic range (P = 0.53) and no difference in total warfarin dose requirement (P = 0.58), 17.9 ± 4.4 mg and 17.1 ± 3.6mg, respectively. Moreover, the CYP2C9 genotype was not a significant predictor of INR within the therapeutic range in the day 6 (P = 0.71).

**Conclusion:** CYP2C9 genotype does not affect the response to warfarin during the first 6 days in Chinese patients.

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**P35**

### LIVER KIDNEY MICROSOMES TYPE 1 ANTIBODIES ARE ASSOCIATED WITH A SIX-FOLD REDUCTION OF THE CYP2D6 ACTIVITY IN PATIENTS WITH CHRON

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Service of Clinical Pharmacology and Toxicology

**Background:** In vitro, Liver Kidney Microsomal type 1 antibodies (LKM-1) have been associated with a reduction of CYP2D6 activity in patients with autoimmune hepatitis type 2.

**Aim:** We considered chronic hepatitis C patients with and without LKM-1 antibodies and investigated whether LKM-1 antibodies were associated with reduced CYP2D6 activity.

**Methods:** All anti-HCV-positive patients enrolled in the Swiss Hepatitis C Cohort Study who had LKM-1 antibodies were compared to a control group of patients without LKM-1 antibodies. Among 2569 patients, 1723 (67.1%) were tested for LKM-1 antibodies. Twenty-three patients (1.3%) had anti-CYP2D6 antibodies that persisted during at least 1-year follow-up. CYP2D6 activity was evaluated by a specific substrate and both groups were genotyped for CYP2D6. CYP2D6 allelic variants were detected by AmpliChip® CYP450 test to exclude individuals with poor metabolizer genotype. Liver insufficiency (INR <0.9 or albumin <35 g/l), concomitant interferon therapy or CYP2D6 inhibitors, ascites, liver transplantation were further exclusion criteria. CYP2D6 activity was assessed with the metabolic ratio dextromethorphan/dextrorphan (DEM/DOR) to classify patients in four phenotypic categories. We compared metabolic activity in both groups and determined the concordance between CYP2D6 activity and CYP2D6 genotype.

**Results:** Among LKM-1 positive HCV patients, ten fulfilled inclusion criteria (mean age 59 years, range 38–77, 60% male) and were included from January 2008 to July 2009. The median CYP2D6 metabolic activity was six-fold lower in LKM-1 positive compared to LKM-1 negative patients (median DEM/DOR ratios 0.096 vs 0.016, P = 0.004). CYP2D6 phenotype predicted from genotype was EM for all patients and controls. Whereas phenotype measured from the DEM/DOR ratio was concordant with predicted phenotype for 70% of LKM-1 negative patient, only 30% of LKM-1 positive patients had a concordant phenotype and 70% showed a phenotypic offset (60% classified as IM and 10% as PM). CYP2D6 activity was not significantly associated with gender, age, BMI, viral genotype, duration since hepatitis C diagnosis and biochemical parameters.

**Conclusion:** CYP2D6 activity drastically decreased in the presence of LKM-1 antibodies. The mechanism and clinical consequences deserve to be further investigated.
P36 CARDIOVASCULAR BIOMARKERS, A USEFUL TOOL IN ANTI-INFLAMMATORY DRUG THERAPY SAFETY MONITORING AND DISEASE MANAGEMENT

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Introduction: Cardiovascular disease continues to be a significant cause of mortality and morbidity among Europeans. Recent epidemiological studies have raised concerns regarding cardiovascular safety of non-steroidal anti-inflammatory drugs (NSAIDs). NSAIDs-use among the general population exceeds 20%, many of whom either have cardiovascular (CV) risk factors or already have a CV disease. The availability of a simple and precise screening marker would be helpful for identifying patients at risk for cardiovascular adverse events (CV-AEs) and for taking the appropriate measures in order to timely prevent adverse drug reactions or worsening of disease.

Materials and Methods: In the present study we searched scientific data-bases (e. g., MEDLINE, ScienceDirect, SpringerLink, Ovid) in order to find studies that have involved CV-AEs monitoring of patients taking NSAIDs and correlations between NSAIDs induced CV adverse drug reactions (CV-ADRs) and variation of plasmatic values of certain markers. Furthermore, we assessed the potential of these markers as screening tests in patients with cardiovascular risk.

Results: We found a total of nine studies in which certain markers had been quantified from plasma and their values correlated with NSAIDs induced CV-ADRs. N-terminal B-type pro-natriuretic peptide (NT-proBNP), the proportion of 11-dehydrothromboxane B2/2,3-donor-6-keto PGF1 (Tx-M/PGI-M) and F2-isoprostanes (F2-IsoPs) were the markers used in order to describe a relationship between NSAIDs use and CV-ADRs. A plasma concentration of >100 ng/l of NT-proBNP is an accurate predictor of CV-ADRs associated with NSAIDs as is an elevated urine Tx-M/PGI-M (>3.45 ± 0.43 ng/mg Cr). Plasma or urine F2-IsoPs could not be accurately correlated with NSAIDs induced CV-ADRs.

Conclusion: The use of specific biomarkers is an important step forward in predicting ADRs to drugs and evaluating patients at risk. Results from the present study will further be used in a case–control study in order to evaluate the predictive capabilities of these specific markers.

P37 EFFECT OF GENETIC POLYMORPHISMS ON THE EFFICACY OF LOW-DOSE AZATHIOPRINE THERAPY IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS


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Introduction: Azathioprine (AZA) has been widely used for organ transplantation and several autoimmune diseases including systemic lupus erythematosus (SLE). The use of AZA is restricted the succession of treatment by adverse drug reactions. Recently, some associations between genetic polymorphisms and clinical efficacy of AZA in SLE patients have been reported. However, the inter-patient variability of clinical efficacy of AZA is not fully explained. This aimed to study was to investigate the effect of genetic polymorphisms on the efficacy of low-dose AZA therapy in patients with SLE.

Patients and Methods: Blood samples were obtained from 50 patients treated with AZA. The polymorphisms of inosine triphosphate pyrophosphatase (ITPA) 94C>A was examined by PCR-RFLP. ITPA 138G>A, xanthine oxidase (XO) 837C>T, XO 2121C>T, XO 3030C>T, inosine monophosphate dehydrogenase 1 (IMPDH) 1575A>G and multidrug-resistance protein 4 (MRP4) 2209G>A were analyzed by Taqman® SNP genotyping assays. The SLE disease activity index (SLEDAI) score was determined to assess SLE activity.

Results: After the low-dose AZA treatment for 1 year, a significant reduction in SLEDAI score was observed in patients with ITPA 94CA or 94AA (P < 0.001), but not in those with ITPA 94CC (P = 0.960). The other polymorphisms showed little contribution to the change in SLEDAI score.

Conclusion: Our study suggested that the patients carrying the ITPA 94A allele showed a significantly better response to low-dose AZA treatment. On the other hand, XO, IMPDH or MRP4 polymorphisms did not have large influence on clinical efficacy of AZA treatment. Additional studies are needed to predict the clinical efficacy of AZA in SLE patients.

P38 NRL972 - A MARKER OF HEPATIC BILIARY FUNCTION: NO INTERACTION WITH PHENOBARBITAL OR ETHANOL

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Background: NRL972 (cholyl-L-lysine-fluorescein) is an investigational diagnostic marker of liver function sensitive to changes in non-metabolic biliary clearance; the elimination of NRL972 is markedly reduced in hepatic cirrhosis and acute hepatitis.

Purpose: To investigate the effects of phenobarbital (PB) and ethanol (ET) on the pharmacokinetics (PK) of NRL972.

Methods: NRL972-PK after single 15-s 2 mg iv-doses were studied on day D01 in 18 male and female healthy subjects according to a three-way cross-over with randomly allocated period-balanced sequences either without pretreatment (R), after 100 mg PB p.o. o.d. from the evening of day D-8 until the evening of Day D-1, or after ET (vodka 40 vol% in orange juice on the evening of D-3 to D-1 [target maximum BAC: 0.75±0.15%] and morning of D01 [target maximum BAC: 1.0±0.15%]).

Results: PB had no effect on the clearance (estimated PB-R: 13 ml/min, 95% CI: -14 to 40) and t½ of NRL972 (estimated PB-R: 0.96, 95% CI: 0.81–1.14); ET also had no effect on clearance (EST-R: 26 ml/min, 95% CI: -3 to 55) and t½ (estimated EST-R: 1.03, 95% CI: 0.91–1.15). All treatments were very well tolerated, except for mild signs of PB- and ET-exposure.

Conclusion: The elimination of NRL972 relies on transporter pathways that are NOT sensitive to interaction with either phenobarbital or ethanol.

P39 EFFECT OF OATP2B1 GENOTYPE (C.935G>A) ON THE PHARMACOKINETICS OF MONTELUKAST IN HEALTHY KOREAN SUBJECTS


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Background and Objective: Recently, it has been reported that montelukast is a substrate of OATP2B1 in vitro. In addition, OATP2B1 polymorphism (c.935G>A) exhibited a substantial effect on pharmacokinetics and pharmacodynamics of montelukast (Mougey et al, 2009, 2010). Montelukast is a leukotriene receptor antagonist (LTRA) that is available as a once-daily oral formulation used to control symptoms associated with asthma in children and adults. In this study, we determined whether polymorphic OATP2B1 genotype influence the pharmacokinetic characteristics of montelukast in healthy Korean subjects.

Methods: Among 200 healthy subjects screened for OATP2B1 genotypes, Twenty-four healthy individuals were recruited according to OATP2B1 genotype (c.935G>A). After administration of a single dose of 10 mg montelukast, plasma concentrations of montelukast were measured using HPLC-MS/MS.
P40

PLASMA 4β-HYDROXYCHOLESTEROL REFLECTS CYP3A4 ACTIVITY IN SUBJECTS TREATED BY MIDOSTAURIN WITH OR WITHOUT RIFAMPINIC

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Introduction: Urinary 6β-hydroxycortisol/cortisol ratio (CR) has been used as a biomarker for CYP3A4 activity. However, some published data showed inconsistencies between CR and midazolam PK changes. Plasma 4β-hydroxycholesterol (4-HC) has recently been suggested as a new reliable biomarker of CYP3A4 activity. The aim of the study is to compare the use of 4-HC and CR in assessing CYP3A4 metabolic activity with and without rifampicin (a potent CYP3A4 inducer) administration.

Patients and Methods: This ancillary study is part of a parallel, placebo-controlled study with subjects randomized to either placebo or rifampicin 600 mg once daily from Day 1–14 with midostaurin (a CYP3A4 substrate) single dose 50 mg on Day 9. Midostaurin PK was assessed with and without rifampicin. Both 4-HC and CR levels were measured on Day 1 (baseline), 6, 9, 11 and 15.

Results: In the presence of rifampicin, midostaurin exposure decreased by approximately 94% from 22799 (CV% = 38.7; placebo) to 1347 (CV% = 46.07; rifampicin) ng/ml. In parallel, 4-HC concentrations and CR increased from 25 to 77 mg/l and 8 to 32, respectively. Moreover, a trend of correlation was observed between midostaurin exposure and 4-HC concentrations among patients in the placebo group which is not seen for CR values (figure).

Conclusion: Both biomarkers can describe major changes of enzyme activities due to induction. But 4-HC can differentiate better CYP3A4 activity differences between subjects, and thus, it can be considered as a more sensitive biomarker for CYP3A4 activity.

The study was supported by Novartis Pharma AG.

P41

NO SIGNIFICANT EFFECT OF SLC01B1 POLYMORPHISM ON THE PHARMACOKINETICS OF URSEDOXYCHOLIC ACID

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Introduction: In a previous study, the fasting plasma concentrations of endogenous ursodeoxycholic acid (UDCA) and its conjugates were found to be affected by SLC01B1 genetic polymorphism in healthy volunteers. In this study, we investigated the effects of SLC01B1 polymorphism on the pharmacokinetics of exogenous UDCA and its metabolites in healthy volunteers.

Methods: In a crossover study with two phases, fifteen healthy volunteers with SLC01B1*1A/*1A genotype, seven with *1B/*1B genotype, and five with *15/*15 or *5/*15 genotype ingested placebo or a single 150-mg dose of UDCA. Plasma concentrations of bile acids and their biosynthesis marker were determined up to 24 h by liquid chromatography-tandem mass spectrometry.

Results: SLC01B1 genotype had no significant effect on the pharmacokinetics of UDCA. The geometric mean ratios (95% confidence interval) of UDCA area under the plasma concentration-time curve from 0 to 12 h (AUC0-12) in subjects with the SLC01B1*1B/*1B genotype and in subjects with the SLC01B1*15/*15 or *5/*15 genotype were 1.07 (0.85, 1.35, P = 0.459) and 0.93 (0.75, 1.15, P = 0.563), respectively. In addition, SLC01B1 polymorphism showed no association with the AUC0-24 of the glycine and taurine conjugates of UDCA, or with endogenous bile acids or the incremental AUC0-24 of a bile acid synthesis marker, either after placebo or UDCA administration.

Conclusions: Genetic polymorphism in SLC01B1 does not affect pharmacokinetics of UDCA, suggesting that OATP1B1 is not rate-limiting to the hepatic uptake of therapeutic UDCA.

P42

CYPIA2 ACTIVITY IN SOUTH ASIANS AND EUROPEANS

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Introduction: The enzyme CYPIA2 plays an important role in the metabolism of medicines including antipsychotics and the detoxification and activation of carcinogens, implicating it in several cancers. CYPIA2 activity demonstrates wide inter-individual variability and inter-ethnic differences. The aim of this study was to investigate CYPIA2 activity in individuals of South Asian (SA) and European (EU) ancestry and explore factors contributing to CYPIA2 variability.

Methods: This was a prospective observational study recruiting healthy males and females of SA (Indian and Sri Lankan) and EU ancestry. CYPIA2 enzyme activity was determined using the validated saliva paraxanthine/caffeine concentration ratio at 4 h following a 100 mg oral dose of caffeine. Saliva samples were analysed using a validated HPLC method. Demographic, clinical and lifestyle information (including dietary habits) was obtained using a questionnaire.

Results: To date 278 participants (113 SA, 165 EU) have completed the study. South Asians had significantly lower CYPIA2 activity (mean ± SEM, 95% CI; 0.46 ± 0.02, 0.42–0.50) than Europeans (0.56 ± 0.28, 0.51–0.60) (P = 0.06). In all subjects smokers had significantly increased CYPIA2 activity (P < 0.01) while women taking oral contraceptives had significantly reduced CYPIA2 activity (P < 0.01). Consumption of 8 or more servings of cruciferous vegetables a week, and more than two cups of coffee daily were associated with increased CYPIA2 activity (P < 0.01). Consumption of six or more servings of apiaceous vegetables decreased CYPIA2 activity (P < 0.01). Discussion Healthy South Asians have lower mean CYPIA2 activity than Europeans and the influence of a number of extrinsic factors has been confirmed.
NRL972 – A MARKER OF HEPATIC BILIARY FUNCTION: INDUCTIVE EFFECTS OF CARBAMAZEPINE

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Background: NRL972 (cholyl-L-lysine-fluorescein) is an investigational diagnostic marker of liver function sensitive to changes in non-metabolic biliary clearance.

Purpose: To investigate the effects of carbamazepine (CBZ) on the pharmacokinetics (PK) of NRL972.

Methods: NRL972-PK after single 15-s 2 mg iv-doses were investigated in 37 young healthy volunteers (19 males) before (D01), after one (D07), two (D14), 3 weeks (D21) of CBZ-treatment (stepwise up to 300 mg b.i.d) and 4 days after the last CBZ-dose (D25). CBZ had to be discontinued prematurely in four subjects due to CBZ-AE.

Results: On average CBZ increased GGT (D01: 17 U/l; D21-D01: +25; CI: 18–32) and alkaline phosphatase (D01: 149 U/l; D21-D01: +16; CI: 8–21), while reducing bilirubin (D01: 10.9 mM; D21-D01: -5.5; CI: -6.9 to -4.2), RBC (D01: 4.69 1012/l; D21-D01: -0.17; CI: -0.27 to -0.08), WBC (D01: 6.4 109/l; D21-D01: -0.8; CI: -1.3 to -0.3), and platelets (D01: 228 109/l; D21-D01: -24; CI: -40 to -8). This was associated with a steeper C (10) to C (30) ratio (D01: 0.22; D21-D01: -0.06; CI: -0.08 to -0.04), faster clearance (D01: 275 ml/min; D21-D01: +92; CI: 69–115), and shorter half-life (D01: 9.1 min; D21-D01: -1.5; CI: -2.1 to -1.0), whereas Cmax was unchanged. Contrasts generally had a statistically significant covariant effect for CBZ. Most effects were already evident after 7 days and had not yet regressed after 4 days (D25).

Conclusion: The elimination of NRL972 relies on transporter pathways that are sensitive to interaction with CBZ. This might reflect CBZ-related MRP2-induction.
VULNERABLE POPULATIONS

P44 MEDICATION OPTIMISATION FOR PATIENTS WITH POLYPHARMACY THROUGH JOINT CONSULTATIONS WITH CLINICAL GERIATRICIAN AND CLINICAL PHARMACIST

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Introduction: This study examined the effectiveness of geriatric day care that includes clinical pharmacist-led medication review in elderly outpatients with polypharmacy and interventions given as an advice to the clinical geriatrician.

Methods: Prospective follow-up study in which included patients were seen at the geriatric day care for medication review, for intervention and baseline measurement (t = 0 months) and 3 months after intervention (t = 3 months). Patients were included if they were >65 years of age and were taking at least five different medications, prescribed by at least two different medical specialists. Medical background and current problems were reviewed by the clinical geriatrician. Medication verification and possible interventions were formulated by the clinical pharmacist. The primary outcome was the alteration in appropriateness of medications between baseline and 3 months after intervention, using the Medication Appropriateness Index (MAI). Secondary outcomes were changes in number of medications, in quality of life (EQ-5D) and in medications mentioned in Beers criteria and in HARM Wrestling.

Results: In 34 patients the average MAI decreased from 28.8 to 15.0 (P < 0.001). The average number of medications decreased from 12.2 to 10.9 (P = 0.011). The number of medications mentioned in HARM Wrestling decreased from 1.59 to 1.19 (P = 0.009). Alterations in quality of life and number of medications mentioned in the Beers criteria were not statistically significant.

Conclusions: The use of a combined healthcare team, consisting of a clinical pharmacist and clinical geriatrician, significantly improves the appropriateness of medication used by elderly patients with polypharmacy. Long term outcome, like the influence on the incidence of adverse events or hospital admission will be further investigated.

P45 RENAL AND DIGESTIVE TOLERANCE OF A FOOD SUPPLEMENT, PHYTALGIC®, IN ELDERLY VOLUNTEERS – A MULTICENTRIC OPEN TRIAL

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It has been shown that Phytalgic®, a food supplement, decreased the need for analgesics and NSAIDs and improved the symptoms of osteoarthritis in volunteers with osteoarthritis (OA) of knee or hip. The aim of the study presented here was to evaluate the renal and digestive tolerance of this food supplement on elderly volunteers (≥70). This was a 6 month multicentric open trial in 50 volunteers with OA. Volunteers took three soft capsules of Phytalgic® (fish oil, vitamin E, Urtica dioica L extract and zinc) per day. ASAT, ALAT, gamma-GT, lipase, amylase concentrations were primary outcome measures characterizing digestive tolerance and MDRD formula for glomerular filtration rate. General biochemical parameters and quality of life by WOMAC function scales were the secondary outcome measures. All these parameters were determined at D0, D42, D84 and D168.

At study inclusion, all biological parameters were within reference values. There was no significant difference of determined parameters throughout the study, concerning renal and digestive tolerability. General biochemical parameters were also not significantly modified. Quality of life evaluated with WOMAC function scales divided in pain, stiffness and incapacity scores was significantly improved in a time-dependent manner as already shown in the efficacy trial realized on this food supplement.

This study confirms the excellent renal and digestive tolerability of Phytalgic® in elderly volunteers. Chronic supplementation with Phytalgic® also improved the symptoms of osteoarthritides and the quality of life.

P46 SAFETY, PHARMACOKINETICS AND OVULATION INDUCTION OF THE FIRST ORALLY ADMINISTERED LOW MOLECULAR WEIGHT L. H AGONIST

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Introduction: Human chorionic gonadotropin (hCG), used to trigger final oocyte maturation in fertility protocols, has several disadvantages including molecular heterogeneity, parenteral administration and long elimination half-life which increases the risk of OHSS. A low molecular weight LH agonist (LH-ago1) has been developed for oral administration. The aim of the first in-human trial was to characterize in safety, tolerability, pharmacokinetics (PK), in part I, as well as to evaluate the pharmacodynamic effect (part II) of LH-ago1 when administered to healthy young sterilized volunteers.

Methods: In part I (randomized, parallel group, double-blind, placebo controlled) seven groups of eight healthy young sterilized women each, received one of the following single doses of LH-ago1: 5, 25, 100, 300, 900, 1800 or 2700 mg or placebo in a 6:2 ratio. The time of dosing was independent of the menstrual cycle phase of the subject. Safety was monitored at regular intervals and serial blood samples were collected up to 96 h post-dose to determine single-dose PK parameters. Thirty healthy young sterilized females from part I continued to participate in part II (open label). Their follicular growth was monitored daily using trans-vaginal ultrasonography (TVUS). When the largest follicle was 15 mm, 0.25 mg ganirelix (Orgalutran®) and 100 IU follitropin beta (Puregon®) were administered. On the next day 0.25 mg ganirelix was given in combination with a single oral dose of LH-ago1. Dose levels for LH-ago1 in part II ranged from 25 mg, to 900 mg. Ganirelix (0.25 mg/day) treatment continued for the next 2 days. Follicular size and follicular collapse were monitored by TVUS and progesterone levels on the 7th day after LH-ago1 treatment were measured to confirm ovulation.

Results: There were no serious adverse events or drop-outs due to adverse events. The majority of adverse events were mild in severity. The PK of LH-ago1 can be characterized by fast absorption (mean tmax 0.5–1 h) and a mean elimination ½ between 30 and 47 h. Across the dose range up to 900 mg, Cmax was dose proportional and exposure (AUC0–∞) was supra-proportional to dose. At higher doses, Cmax and AUC0–∞ tended to reach a plateau. TVUS measurements and progesterone data showed that LH-ago1 induced an ovulation within 2 days after dosing. This effect started at 100 mg and reached its maximum effect at the 300 and 900 mg dose.

Conclusions: After single oral doses up to 2700 mg, the low molecular weight LH agonist is safe and well tolerated, displays non-linear PK and is able to successfully induce ovulation in women in doses between 300 and 900 mg.
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IMPACT OF NON-ADHERENCE TO ANTIRETROVIRAL THERAPY IN HIV-INFECTED CHILDREN
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Introduction: Data exist showing failure of treatment with antiretrovirals due to inadequate adherence to the prescribed dosing regimen. Several studies have been performed to assess whether high rates of adherence are necessary to achieve and maintain viral suppression during the course of therapy [1, 2, 3]. However, none of these studies have explored compliance in a systematic manner, identifying which drugs are more likely to be affected by poor adherence. The aim of this investigation was to therefore evaluate the forgiveness of antiretroviral therapy to variable compliance, taking into account the differences in pharmacokinetics and pharmacodynamic properties of currently used drugs.

Methods: Simulation scenarios were evaluated using a hypothetic population of HIV-infected children (n = 100) aged between 3 months and 11 years. Published pharmacokinetic and pharmacodynamic models were integrated with an established model for viral replication to predict treatment outcome based on different degrees of adherence to therapy for each class of drugs used in first-line therapy (non-nucleoside reverse transcriptase inhibitors and nucleoside reverse transcriptase inhibitors). The measures of interest were viral load and CD4 cell count.

Results: Preliminary results suggest that efavirenz, a non-nucleoside reverse transcriptase inhibitor with long half-life and high potency, allows for variable quality of compliance, such as delays in drug administration, whilst it is more susceptible to interruption of therapy for long periods (2–3 weeks).

Conclusions: Despite its relevance in therapeutic applications, the implications of poor compliance and most importantly the degree of forgiveness of antiretrovirals has not been assessed in a quantitative manner. Our results show that simulations can be applied as a tool to explore non-adherence to treatment. The use of this model-based approach provides a framework for the optimisation of the dosing regimens for antiretroviral drugs, unravelling the set of pharmacokinetic and pharmacodynamic properties that are required for forgiveness.

References:

P48
THE EFFECT OF VITAMIN E ON HOT FLASHES IN MENOPAUSAL WOMEN
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Introduction: Menopause is one of the steps in women’s life which accompany with a lot of problems. One of the most common problems of menopause is hot flashes which affect about 75% of menopausal women in the world. Conventional treatments that suggested for hot flashes such as hormone replacement therapy (HRT), anti-depressant drugs, α-adrenergic agonist drugs and herbal therapies were not accepted by women due to the side effect or ineffectiveness. This study was undertaken to assess the effect of vitamin E on hot flashes in menopausal women.

Methods: Sixty menopause women suffering from hot flashes were selected and divided into two groups using randomized sampling method and placebo-controlled cross over clinical trial was conducted. After 1 week baseline period, the first group received 400 IU vitamin E daily while the other group received placebo for 4 weeks. In order to eliminate the carry over effect of cross over trial, 1 week washout was considered. Then the drugs were reversed for each group and the study was continuing for another 4 weeks. In each group using diary, hot flash frequency and severity before the treatment, after the first and second period of treatment was measured and compared with each others.

Results: The results show that in the first group vitamin E and placebo caused a decrease of 60% and 31% in hot flash frequency, respectively and for the second group these values were 49% and 26%, respectively. The effect of vitamin E and placebo on hot flash severity were also highly significant in both groups. Furthermore, the evaluation of the results indicated that the effect of vitamin E compare to placebo on alleviating of hot flash symptoms was more significant.

Conclusion: Based on our trial, vitamin E is recommended for the treatment of hot flashes in menopausal women.

Key words: vitamin E, hot flashes, menopause.

P49
CLINICAL TRIALS DURING PREGNANCY: WHAT HAS BEEN DONE. A SYSTEMATIC REVIEW
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Introduction: The development of a drug rarely includes pregnant women in clinical trials. Consequently, most drugs are subject to contraindications and warnings because of a lack of relevant information for pregnancy. The only available data to provide scientific evidence for rational therapeutic decisions in pregnant women come from observational studies. Clinical research in pregnancy is limited and there is an ongoing debate about the regulatory and ethical aspects of including this population in clinical trials. Our main objective was to describe clinical trials conducted in pregnant women over the past decade.

Materials and Methods: We performed a systematic review of clinical trials published over the past 10 years. The analysis included: type of disease, intervention, gestational age at inclusion and during intervention, main objectives and other relevant methodological issues. We searched the PubMed database for studies with the preferred terms ‘pregnancy’, ‘human’, and ‘clinical trials’ published between 01/01/2000 and 31/12/2009.

Results: Out of 1264 publications, 762 (60%) were excluded due to the fact that they were observational studies (566) or did not include pregnant women (196), leaving 502 for analysis. Of these, 53% were maternal or fetal preventive studies and 47% were therapeutic trials focused in current obstetric diseases (59%), chronic conditions (21%), intercurrent diseases (11%), and perinatal diseases (9%). As regards to the type of intervention, 66% were pharmacological, 10% lifestyle-related (including exercise), 6% nutritional, 6% surgical and 4% diagnostic examinations. Of the studied drugs, 16% were for labour induction and 15% for abortive procedures, followed by multivitamins and micronutrients (14%), labour analgesia and anaesthesia (12%), antibiotics (6%), tocolytics (5%) and antimalarial drugs (4%). The main objectives were efficacy (55%), effectiveness (5%), safety (4%), pharmacokinetics & pharmacodynamics (3%). Thirty-four had combined objectives. Eighty-one percent of the studies were controlled, randomized and parallel-design trials; 19% were blinded.

Conclusion: This systematic review shows that clinical trials in pregnant women are mainly conducted with an efficacy objective, in maternal-fetal prevention and in obstetric diseases, to study labour induction and abortive measures. This is in line with the types of intervention and drugs involved. Additional studies are needed to assess needs in pregnancy prescription.
P50
ANTICHOLINERGIC RISK SCALE SCORE AND PHYSICAL FUNCTION IN OLDER HOSPITALISED PATIENTS: IMPLICATIONS FOR ADVERSE OUTCOMES

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Background: The anticholinergic risk scale (ARS) score is associated with the number of anticholinergic side effects in older outpatients. We speculated that higher ARS scores are also associated with reduced physical function (Barthel Index, primary outcome) and predict adverse outcomes in older hospitalised patients.

Methods: Clinical and demographic characteristics, Barthel Index, full medication exposure and ARS score on admission were recorded in 362 consecutive patients (age 83.6 ± 6.6 years) admitted to two geriatric units (Aberdeen, NHS Grampian) between February 1, 2010 and June 30, 2010. Data on length of stay (LOS) and in-hospital mortality (secondary outcomes) were obtained from electronic records.

Results: After adjusting for age, gender, dementia, institutionalisation, Charlson Co-morbidity Index, admission site, and number of non-anticholinergic drugs, a unit increase in ARS score was associated with a 29% reduction in the odds of being in a higher Barthel quartile than a lower quartile (OR 0.71, 95%CI 0.59–0.86, P = 0.001). The Barthel components mostly affected were bathing (P < 0.001), grooming (P = 0.001), dressing (P < 0.001), bladder function (P = 0.011), transfers (P = 0.005), mobility (P < 0.001), and stairs (P = 0.001). Higher ARS scores predicted in-hospital mortality amongst patients with hyponatremia (HR 3.66, 95% CI 1.70–7.89, P = 0.001), but not those without hyponatremia (HR 1.04, 95% CI 0.70–1.54, P = 0.86). The ARS score did not significantly predict LOS (HR 1.02, 95%CI 0.88–1.17, P = 0.82).

Conclusions: High ARS scores on admission are negatively associated with various components of the Barthel Index and predict in-hospital mortality in the presence of hyponatremia amongst older patients. The ARS score may be useful in the acute setting to improve risk stratification.

P51
AVAILABILITY OF INFORMATION ABOUT OLD PERSONS IN EUROPEAN PUBLIC ASSESSMENT REPORTS AND SUMMARIES OF PRODUCT CHARACTERISTICS

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Introduction: Health care professionals can find information about a medicine in the summaries of product characteristics (SmPCs) and the European public assessment reports (EPARs). The SmPC contains the claims of the application holder and is often read by health care professionals; the EPAR describes the regulatory considerations. Older persons are frequently excluded from registration studies in order to increase internal study validity.1,2 The ICH E7 guideline on geriatrics has been introduced to enhance the inclusion of older persons in these studies. Three The aim of this study was to evaluate the availability of information about how to use an authorised medicine in older persons in the EPARs and SmPCs of recently approved medicines within the EU.

Methods: We selected 26 SmPCs and EPARs of new chemical entities and/or complete and independent applications of systemically administered drugs for a geriatric indication with a first European marketing authorisation between 2008 and 2011. We used the ICH E7 guideline categorised in four topics, containing nineteen items (see table 1), to evaluate the availability of information in both source documents.

Results: The medicines were approved for various therapeutic indications. More detailed results are shown in Table 1.

<table>
<thead>
<tr>
<th>Table 1. Availability of information in the SmPCs and EPARs</th>
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<tr>
<td>Drugs (n = 26)</td>
</tr>
<tr>
<td>SmPC (%)</td>
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<tr>
<td>Definition of the population (n = 4)</td>
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<tr>
<td>Clinical experience (n = 4)</td>
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<tr>
<td>PK studies (n = 8)</td>
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<tr>
<td>Drug-drug interaction studies (n = 3)</td>
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<tr>
<td>Overall (n = 19)</td>
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Conclusions: Our study shows that the information about using a medicine in old persons in the SmPCs and EPARs, according to the ICH E7, is incomplete, with 43% and 58% respectively. The awareness of the need for adequate participation of old persons in clinical studies is increasing.2, 4, 5 This study underpins the perspective that more information to guide geriatric prescribing should be covered in the SmPCs and EPARs.


P52
EFFECTS OF RENAL DYSFUNCTION ON THE PHARMACOKINETICS OF NRL972, A MARKER OF HEPATIC BILIARY FUNCTION

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Background: NRL972 (cholyl-L-lysine-fluorescein) is an investigational diagnostic marker of liver function sensitive to changes in non-metabolic biliary clearance; the elimination of NRL972 is markedly reduced in hepatic cirrhosis and acute hepatitis.

Purpose: To investigate the effects of severe (SRI: CLCr <30 ml/min per 1.73 sqm BSA; N: 16) and mild-to-moderate renal dysfunction (MRI: 30 ≤ CLCr <90 ml/min; N:20) on the pharmacokinetics (PK) of NRL972 relative to matched controls (CON: CLCr >90 ml/min; 15).

Methods: Plasma and urinary PK of NRL972 after single 15-second 2 mg iv-doses.

Results: SRI caused a slight enhancement of the elimination of NRL972 as shown by the C(30)/C(10)-recovery (estimated SRI-CON: -0.11; 95% CI: -0.19 to -0.03) and clearance CL (SRI-CON: 110 ml/min; N: 16) and mild-to-moderate renal dysfunction (MRI: 30 ≤ CLCr <90 ml/min; N:20) on the pharmacokinetics (PK) of NRL972 relative to matched controls (CON: CLCr >90 ml/min; 15).

Conclusion: Renal dysfunction does not impair the elimination of NRL972; instead, there is a trend of enhanced NRL972-disposition in patients with compromised renal function.
Higher total number of prescribed drugs was found in the group of users with cholinergic medications. Their occurrence at the time of hospital admission was related to the increased sensitivity of older patients to anticholinergic medications. Therefore the aim of the present study was to evaluate the use of anticholinergic medications in elderly patients and to identify risk factors which increase the probability of prescription of such drugs.

**Introduction:**
As a part of the pharmacokinetic (PK) study on 2 g intravenous (iv) loading dose paracetamol in pregnant women, intra-subject (pregnant vs non-pregnant state) PK differences were investigated.

**Patients and Methods:**
Following informed consent, a subgroup of six pregnant women who underwent a cesarean section and received a 2 g loading dose of iv paracetamol were admitted again for the same loading dose administration scheduled for at least 10 weeks after the pregnancy. At both visits, blood samples were collected at the same predetermined time points (1, 2, 4 and 6 h after drug administration). A one-compartmental linear PK model in a naïve pooled approach was used. Paired data were compared using nonparametric Wilcoxon signed-rank test. Clinical data were reported as median (range).

**Results:**
Forty-eight plasma concentration-time points were collected in six women. Median (range) age was 32 (27–37), gestational age 38 (33–39) weeks, postpartum week 11.2 (10.7–15). When observations in pregnancy were compared to non-pregnant state, median (range) Cmax [23.06 (13.88–32.32) vs 64.5 (38.49–75.75) mg/l] were significantly lower during pregnancy (P = 0.0312) while no difference was observed in Ctrough [4.08 (2.88–8.17) vs 5.73 (2.66–11.88) mg/l, P = 0.0625].

Naïve pooled paracetamol half-lives were 1.99 vs. 2.13 h, clearances 28.73 vs. 21.20 l/h and distribution volumes 82.34 vs 65.26 l in pregnant vs non-pregnant state respectively.

**Conclusion:**
Following an iv paracetamol loading dose, pharmacokinetic intra-individual differences between pregnant and non-pregnant state were in line with differences observed between pregnant women and healthy volunteers. These pharmacokinetic estimates might be of pharmacodynamic relevance.

**P53**
**DOES PREGNANCY AFFECTS INTRA VENOUS PARACETAMOL DISPOSITION? A PAIRED ANALYSIS**

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**Introduction:**
As a part of the pharmacokinetic (PK) study on 2 g intravenous (iv) loading dose paracetamol in pregnant women, intra-subject (pregnant vs non-pregnant state) PK differences were investigated.

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**Conclusion:**
Following an iv paracetamol loading dose, pharmacokinetic intra-individual differences between pregnant and non-pregnant state were in line with differences observed between pregnant women and healthy volunteers. These pharmacokinetic estimates might be of pharmacodynamic relevance.

**P54**
**MEDICATIONS WITH ANTICHOLINERGIC EFFECTS AND THEIR USE IN ELDERLY PATIENTS**

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**Introduction:**
Medications with anticholinergic effects may commonly cause unrecognised adverse drug reactions. Changes in pharmacokinetics of drugs and age-related decline in cholinergic neurotransmission, contribute to the increased sensitivity of older patients to anticholinergic effects. Therefore the aim of the present study was to evaluate the use of anticholinergic medications in elderly patients and to identify risk factors which increase the probability of prescription of such drugs.

**Methods:**
The study was carried out on a sample of 1636 patients aged 65 years hospitalised in three municipal hospitals. The most important risk factors influencing the use of anticholinergic medications were identified using the binary logistic regression model.

**Results:**
Hospitalisation led to a significant increase in the use of anticholinergic medications. Their occurrence at the time of hospital admission and discharge was 10.5% and 14.2%, respectively (P < 0.001). A higher total number of prescribed drugs was found in the group of users compared to non-users, at both hospital admission (7.2 ± 3.5 vs. 5.7 ± 3.1; P < 0.001) and discharge (8.7 ± 3.1 vs. 7.5 ± 2.9; P < 0.001). Constipation, urinary incontinence and retention, immobilisation, gastroduodenal ulcer disease, depression, Parkinson’s disease and epilepsy were found to be the most important risk factors for prescription of anticholinergic medications.

**Conclusion:**
In patients with the presence of risk factors mentioned above, the active search for drugs with anticholinergic properties is necessary in order to reduce the patients’ anticholinergic burden. This study was supported by grant VEGA 1/0135/09.
in the thyroid gland. Nuclear medicine specialist also encouraged continuing the pregnancy.

We contacted patient and found she delivered a healthy full term boy with no evidence of fetal hypothyroidism at birth. After 6 months we contacted mother again, and the baby is doing well, normally developing without any motoric or intelectual impairment.

Results: During the study period, 43 patients were included and none of them received the wrong type of EN, but 2.3% received insufficient amounts of calories (53% reduction over 2009). The technique of administration was not correct in 21% of the patients (32% reduction) although in any case EN was transferred to a container (100% reduction). Medication was incorrectly administered in 16% of patients (30% reduction).

Conclusions: Training sessions given by the Pharmacy and Endocrine Services have reduced errors associated with the administration of EN and drugs in patients with NGT and PEG, but it is still necessary to improve in the tube composition (polyurethane or silicone) and administration of water through the tubes, to ensure adequate hydration of patients and proper maintenance of tubes.

P57
EVALUATION OF PARENTERAL NUTRITION SENDING TO HOSPITAL SERVICES AND NURSING KNOWLEDGE RELATED TO THEIR ADMINISTRATION

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Introduction: Parenteral nutrition (PN) order forms are send to Pharmacy Service in the morning and they are evaluated and validated by the pharmacist. PN are prepared in the afternoon and Hospital Services receive them at 19 pm approximately. If a PN finish before arriving the next one, infusion should be maintained with glucose 10%.

Objective: To evaluate PN sending and nursing knowledge related to their administration.

Methods: In November 2009, an anonymous survey was completed by nursing staff of our Hospital. The survey consists of five questions with two possible answers: ‘yes’ or ‘no’ and it was distributed to 138 nurses. In addition, they were asked about length of experience and about Service they are working at.

Results: Ninety-seven surveys (70%) were completed and the results were:
1. 96% of nurses considered that the label of PN express all necessary information.
2. 73% answered that PN are received on time.
3. 67% knew standardized PN in the Hospital and the different routes of administration.
4. 89% of nurses considered that they know what to do if a PN finish before arriving the next one, but after analysing the responses we found that only 45% really know what to do.
5. 95% answered that it should be necessary to receive training sessions about PN administration.

Conclusions: Pharmacy Service label PN with complete information, ensuring adequate identification, but should improve the sending time to Hospital Services. Knowledge about PN administration increases with experience, although it would be necessary to conduct training sessions for nursing staff.

P59
COMPARISON OF 7.5% NaCl/6% DEXTRAN 70 AND 0.9% NaCl ON SERUM SODIUM LEVELS IN PATIENTS UNDERGOING TRANSURETHRAL RESECTIOM OF PROSTATE

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1Department of Anesthesia, Selcuk University, Konya, Turkey; 2Department of Pharmacology, Selcuk University, Konya, Turkey

Introduction: Hyponatremia as a result of hemodilution caused by absorption of hypotonic irrigation fluids is the leading cause of mortality and morbidity in patients undergoing transurethral resection of the prostate (TURP). 0.9% NaCl (NS) is usually the routine fluid preloading fluid for spinal anesthesia. The aim of this prospective randomized study was to compare the effects of small volume 7.5% NaCl/6% dextran 70 (HSD) with NS solutions regarding serum sodium (Na+) levels in patients undergoing TURP under spinal anesthesia.

Methods: After institutional approval and informed consent, 60 patients were randomized. Group HSD (n = 30) received 2 ml/kg of 7.5% NaCl/6% dextran 70, while group NS (n = 30) received 7 ml/kg of 0.9% NaCl solution as preloading. Spinal anesthesia was performed with 2 ml of 5% hyperbaric bupivacaine+0.5 ml of fentanyl. Serum sodium levels were measured before and after the operation. Hemodynamic data and side effects were also recorded. Student’s t and Mann–Whitney–U tests were used as appropriate P < 0.05 was considered as significant.

Results: Preoperative Na+ levels and irrigation volumes were similar. Small volume HSD resulted in higher postoperative Na+ levels.

Table 1: Serum Na+ levels

<table>
<thead>
<tr>
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<th>HSD</th>
<th>NS</th>
<th>P</th>
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<tr>
<td>Preoperative Na+</td>
<td>141.0 ± 3.2</td>
<td>142.3 ± 3.2</td>
<td>0.106</td>
</tr>
<tr>
<td>Postoperative Na+</td>
<td>145.1 ± 3.1</td>
<td>141.6 ± 2.9</td>
<td>0.000*</td>
</tr>
<tr>
<td>Irrigation fluid (ml)</td>
<td>12000.0 ± 4601.3</td>
<td>11566.6 ± 3114.8</td>
<td>0.67</td>
</tr>
<tr>
<td>*P &lt; 0.05.</td>
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</table>

Conclusions: This study shows that small volume HSD is more effective in preventing hyponatremia than NS in patients undergoing TURP with spinal anesthesia.

P58
ANALYSIS OF THE IMPACT OF TRAINING SESSIONS ON THE IMPROVEMENT OF ENTERAL NUTRITION AND DRUG ADMINISTRATION THROUGH NASOGASTRIC

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Introduction: During the months of June, July and August 2009, errors in the administration of enteral nutrition (EN) and drugs in patients with nasogastric tube (NGT) or percutaneous endoscopic gastrostomy (PEG) were analyzed in our Hospital. The Pharmacy Service and Endocrine Service decided to conduct a training session about enteral nutrition for nursing and auxiliary staff.

Objective: To analyze the impact of training sessions to nurses and auxiliary staff in relation to enteral nutrition (EN) and drugs administration.

Methods: All patients receiving EN between 1st August and 31st October 2010 were included and interventions made by clinical pharmacists were evaluated. The results were compared with data prior to the training sessions.

Results: Ninety-seven surveys (70%) were completed and the results were:
1. 96% of nurses considered that the label of PN express all necessary information.
2. 73% answered that PN are received on time.
3. 67% knew standardized PN in the Hospital and the different routes of administration.
4. 89% of nurses considered that they know what to do if a PN finish before arriving the next one, but after analysing the responses we found that only 45% really know what to do.
5. 95% answered that it should be necessary to receive training sessions about PN administration.

Conclusions: Pharmacy Service label PN with complete information, ensuring adequate identification, but should improve the sending time to Hospital Services. Knowledge about PN administration increases with experience, although it would be necessary to conduct training sessions for nursing staff.

P60
DRUG BURDEN INDEX (DBI) ASSOCIATED WITH FUNCTION IN OLDER PEOPLE LIVING IN FINLAND

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Clinical Nutrition, Department of Geriatrics, University of Eastern Finland, Finland; 5Leppävirta Health Centre, Leppävirta, Finland

Introduction: The DBI, an evidence based tool used to capture a person’s total exposure to anticholinergic and sedative drugs has been associated with impaired function in older people living in US and Australia. The aim of this study was to investigate the relationship between the DBI and functional outcomes in older people enrolled in the Geriatric Multidisciplinary Strategy for the Good Care of the Elderly (GeMS) study, Finland.

Methods: This was a randomised comparative study including community-dwelling people aged ≥75 years. Outcomes included 10 m-walking speed, chair stands test, timed up and go (TUG) test, grip strength, instrumental activities of daily living (IADL) and Barthel Index.

Results: The study population consisted of 700 participants. The mean age (SD) of the population was 81.3 ± 4.6 years, and 69% were female. Exposure to DBI drugs was identified in 37% of participants: 24% had a DBI range between >0 < 1, and 13% DBI ≥ 1. After adjusting for covariates, exposure to DBI drugs was associated with slower walking speed (P < 0.0001), poorer performance on chair stands (P = 0.0001) and TUG (P < 0.0001), difficulties in IADL (P < 0.0001) and Barthel Index (P < 0.0001). The mean adjusted walking speed, time to complete chair stands and TUG, IADL and Barthel scores were significantly poorer among participants with higher DBI ranges.

Conclusions: In older community-dwelling adults living in Finland, DBI was associated with impaired physical performance. This finding supports the use of the DBI tool to measure the impact of medications on function in older people.

P61
LUTEIN SUPPLEMENTATION INCREASES MACULAR OPTICAL DENSITY IN PATIENTS WITH AGE-RELATED MACULAR DEGENERATION

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Introduction: Several large scale studies indicate that reduced intake of lutein, a major component of the macular pigment, is a risk factor for the development of age-related macular degeneration (AMD). The purpose of the present study was to test the hypothesis that lutein supplementation increases macular pigment optical density (MPOD). Additionally, we investigated whether lutein supplementation improves visual acuity (VA) and macular function (mean differential light threshold, MDLT). 

Methods: In this randomized (2:1), placebo-controlled, double-masked, parallel group study, 126 patients with AMD (AREDS stages 2, 3 and 4) received either lutein or placebo for 6 months. MPOD was measured using a custom-built reflectometer. VA was assessed with ETDRS charts and MDLT was assessed using a microrimeter.

Results: Lutein intake significantly increased MPOD by 27.9 ± 2.9% (P < 0.001 vs. placebo). A tendency towards an increase was seen for MDLT and VA (MDLT: P = 0.096 vs. placebo, VA: P = 0.070 vs. placebo). After 6 months a significant correlation was found between the increase in MPOD and the increase in MDLT (r = 0.25, P = 0.027) as well as between the increase in MPOD and the increase in VA (r = 0.27, P = 0.013).

Conclusions: The present study demonstrates that lutein supplementation increases MPOD as assessed with an objective method. The correlation between the change in MPOD and the change in VA and MDLT indicates that patients who show a pronounced increase in MPOD also benefit in terms of visual function. An unrestricted research grant from Pharmaselect is thankfully acknowledged.
**PHARMACOVIGILANCE**

**P63**

**MOST FREQUENT MEDICAL ERRORS IN CRIMEA, UKRAINE**

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One of the most important factors leading to increase of drug side effects frequency is medical errors. We analyzed a structure of medical errors in Autonomic republic of Crimea in period from 2000 to 2010 year. In our work we used 5303 drug adverse effect reports sent by doctors to Crimea department of State Pharmacological center of Ministry of health of Ukraine. It was found that most frequent type of medical errors (3.8% of all reports) in region is an ignoring of drug allergy anamnesis: doctors prescribed the drug caused reaction in past or drug that belongs to the same pharmacological group. In 0.4% of cases we registered development of severe bronchial constriction in patients with bronchial asthma caused by non-steroidal anti-inflammatory drugs such as diclofenac sodium, metamisol sodium and ketorolac. Although the efforts of State pharmacological center in regulation of dextrane use they continue to be prescribed out-of-label, doctors do not carry out test for sensitivity and do not administer specific antidotes blocking dextrane antibodies. In last years 4 lethal cases caused by dextrans were registered in Ukraine, one in Crimea. A serious medical error in pediatrics is administration of two and more antipyretics for same patient (0.7%) and use of paracetamol as medicine preventing fever (0.2%). In our opinion the main reasons of mentioned above errors are shortage of knowledge of numerous trade names of drugs which contain same active substance and nonrational use of drugs particularly caused by pharmaceutic manufacturers’ pressing on doctors.

**P64**

**ANALYSIS OF FREQUENCY AND STRUCTURE OF ADVERSE REACTIONS FROM ORAL ANTICOAGULANTS, RECORDED BY SPONTANEOUS REPORTING IN RUSSIA**

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**Introduction:** Currently, thrombotic complications are one of the leading causes of cardiovascular mortality, morbidity and hospitalization, bringing huge economic losses to the state. To reduce the risk of such complications, patients are assigned oral anticoagulants (OAC), however, and these drugs in turn are the cause of many complications in the form of adverse drug reactions (ADR). For the first time in the Russian Federation analyzed ADR frequency and structure by using OAC in clinical practice, reported by spontaneous reporting.

**Materials and Methods:** ADR analysis of frequency and structure from oral anticoagulants, reported by spontaneous reporting was carried out using an electronic database of Roszdravnadzor, with the last update in October 1, 2010 (http://www.roszdravnadzor.ru/). Carried out the analysis contained in the database of spontaneous reports of ADPfór 3 registered in the Russian Federation OAK: Warfarin, Aacenocoumarol (Sinkumar), Fenidinomum. We studied the frequency and structure of adverse drug reactions by type, by specification, by severity and by outcome). To assess the causal relationship ‘drug-ADR’ was used Naranzho scale.

**Results:** In conducting the analysis contained in the database Roszdravnadzor as of October 1, 2010, spontaneous reports of ADR by using Warfarin, Aacenocoumarol (Sinkumar), Fenidinomum was revealed: 82 reports of ADR. With all this in the Roszdravnadzor database as of October 1, 2010 contained 13,497 spontaneous reports of all ADR. Thus, the share of the ADR by using oral anticoagulants in the overall structure of all ADR is 0.61%.

Of the 82 cases of ADR from oral coaguants, were seriously 61 cases. Totally in the database registered 6581 cases of serious ADR from the use of all drugs. Thus, the percentage of oral anticoagulants ADR to all drugs ADR is 0.9%.

Total observed three deaths from the use of oral anticoagulants. In total, 235 registered deaths by all drugs. Consequently, the percentage of deaths from oral anticoagulants in the database amounts to 1.3%.

**Conclusions:** According to the FDA, oral anticoagulants ADR, took 3rd place by frequency and death reason among all major ADR from all drugs. We obtained the low incidence of oral anticoagulants ADR in Roszdravnadzor database by following reasons: apparently not adequate statistics cover of ADR and particularly bleedings (especially ‘serious’) from oral anticoagulants can ‘hide’ under other diagnoses (as a complication of peptic ulcer or hemorrhagic stroke by type, etc. etc.). That’s why, the results of FDA and Roszdravnadzor are such different, but it doesn’t mean that the problem in Russia isn’t serious.

**P65**

**PHARMACO-TOXICOLOGICAL EVALUATION OF DRUG IMPURITIES, BENEFIT/RISK ASSESSMENTS AND OPTIMISING THE SAFE USE OF MEDICINES-ROLE OF CLINICAL PHARMACOLOGIST**

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A good benefit/risk assessment is vital to ensuring new drugs are made available as early as possible without undue risks to patients. In some cases, early availability could be achieved through a ‘staggered approval’ system, where the drug is initially approved for a better defined or more restricted population, and then broadened to a wider population when more ‘real-life’ data are available. The drug licensing process and the relative effectiveness/cost/benefit process are two separate things especially related to genetic drugs. Efficacy vs. effectiveness, relative efficacy of generics vs. originator, can differ in terms of clinical endpoints.

Regulators – pharmaceutical technology assessment experts are faced more frequently with different manufacturing approaches to the same product. Ordinary impurities are defined as those species in drug substances and/or drug products that have no significant, undesirable biological activity in the amount present. But these impurities may arise out of synthesis, preparation or degradation of compendial articles. In certain instances, impurities that pose a potential health risk may be detected. Potential API impurities depend on source (plant, animal, microbial) and manufacturing process for enzymes and include proteins (enzyme and host cell), nucleic acids, endotoxins and bioburden.

Risk Assessment Framework is Based on three Principal: Control of incoming materials, Process understanding and analytical surveillance of API and Safety evaluation of potential residues.

It is important to stress out that inherent differences exist between small molecule impurities and enzyme preparation-related residues. Risk assessment is based on a scientific evaluation of relevant safety and process risk factors. Process risk factors as enzyme source, stage in synthesis can influence clinical risk factors. Clinical risk factors are related to dosing route/oral, parenteral, inhalation, ocular, dermal that could be followed with hypersensitivity reactions/, limited dosing duration, and lower dose-response relationships that often could not be evident on the basis of evaluation submitted documentation in process of registration especially when it is generic drug.

These significant intra-individual differences in responses could be detected only after putting drug on the market.
Physicians’ awareness of adverse drug reactions

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**Introduction:** Adverse drug reactions (ADRs) pose a significant medical problem. Comprehensive knowledge of potential ADRs by physicians assures safe drug therapy. The aim of the study was to investigate physicians’ awareness of ADRs.

**Materials and Methods:** An anonymous survey of Russian-speaking physicians was performed through 2006–2011 with the use of a web-based questionnaire regarding ADRs for visitors of www.antibiotic.ru website.

**Results:** In total 146 respondents participated in this survey. Most of participants (121/146 – 81.2%) were employees of inpatient departments. There were 56 (38.6%) surgeons; 32 (22.1%) specialists in clinical pharmacology; 23 (15.9%) internists and 10 (6.9%) pediatricians. Professional experience of >10 years had 68 (46.6%) of respondents; 5–10 years – 42 (28.8%) and <5 years – 36 (24.7%) participants. Most of responders (82.9%) esteem ADRs as a substantial problem and 95.9% of physicians encountered with ADRs in their practice. ADRs were caused by antibiotics – 38.4%, nonsteroidal anti-inflammatory drugs (NSAIDs) – 19.9%, antihypertensives – 9.7%, glucocorticoids – 8.7%, sedatives – 5.4%, digitalis glycosides – 4.5%, diuretics – 3.6% and others – 9.7%. Clinical manifestations of ADRs involved skin (27.3%), gastrointestinal tract (24.3%), respiratory system (10.6%), blood (10.3%), central nervous system (5.0%), multiple organ dysfunction syndrome (7.8%), cardiovascular (6.9%) and urinary system (3.9%).

Serious adverse events (SAEs) were diagnosed by 59 (42.1%) physicians. In most cases SAEs were reported by clinical pharmacologists (32.2%), surgeons (28.8%) and internists (16.9%). SAEs were caused by antibiotics in 28.4%, NSAIDs in 18.9%, contrast media in 9.5%, glucocorticoids in 5.4% and antihypertensives in 4.1% of cases. ADRs were obligatory reported in source documents by 82 (58.6%) respondents, occasionally – by 55 (39.2%) and never – by 2 (1.4%) respondents. The reported reasons for opt SAEs reporting were lack of awareness (37.5%), lack of time (18.8%), willingness to avoid administrative investigation of the issue (18.8%), considering ADR as non-significant (6.3%) and a feeling of incompetence (6.3%). Physicians working at outpatient clinics reported only 57.8% of noted ADRs, while in hospitals 72.7% were reported.

**Conclusion:** Most of physicians acknowledge frequent occurrence of ADRs in clinical practice. Antibiotics and NSAIDs are the most common causes of ADRs. ADRs involving skin and gastrointestinal tract account for >50% of all ADRs.

Source of the funding – grant.

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**P67**

**CONSTRAINTS IN SCREENING FOR CLINICAL LABORATORY ADVERSE EVENTS IN PHASE I TRIALS**

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**Background:** AE-attrition of on-study changes in clinical laboratory test results (CLINLAB) is subject to several constraints.

**Purpose:** (i) to propose a systematic procedure to identify and qualify CLINLAB-AE, and (ii) to draw attention on confounding on-study medication-unrelated factors resulting in relevant CLINLAB-AE.

**Methods:** To process CLINLAB-results via the following steps: (i) compare vs. CLINLAB reference range (= identify anomalies), (ii) grade anomalies vs. CTCAE-4 criteria (= identify and qualify noteworthy values), (iii) cross-check on-study changes vs. reference database (= identify noteworthy changes), (iv) identify noteworthy changes leading to noteworthy values/anomalies, and (v) overall evaluation by the investigator.

**Results:** This approach may be confounded by the occurrence of unexplained substantial on-study CLINLAB-fluctuations that are not medication-related (while also occurring under placebo). We demonstrate such interferences for haemoglobin, liver enzymes and triglycerides, mainly observed in phase I trials that require 2–3 weeks in-house hospitalisation with repeated PK- and CLINLAB-testing.

**Conclusion:** The chosen multistep approach provides systematic, objective and robust analysis of CLINLAB-data and identification of CLINLAB-AE, unconfounded by inconsistencies in the investigator’s judgment. However, this might still be confounded by unexplained on-study medication-unrelated CLINLAB-fluctuations. There is need to create more awareness and to build a broader reference database in order to avoid that such observations are seen as an indication of poor study conduct.

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**P68**

**DIPYRIDAMOLE THERAPY AFTER STROKE: ADVERSE EFFECTS ARE COMMON**

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**Background:** The antiplatelet clopidogrel has equivalent efficacy to aspirin with dipyridamole as secondary prevention after stroke. Randomised controlled trials suggest dipyridamole has more limited tolerability and its dosing is less convenient. We assessed the number of patients attending a cerebrovascular clinic who had been prescribed dipyridamole and the proportion who had experienced adverse effects.

**Method:** We conducted a prospective analysis of patients attending a cerebrovascular clinic between 16/01/08–30/01/08, 07/07/08–04/08/08 and 12/01/09–30/01/09 after stroke. Hospital records were reviewed to identify demographics. Patients who had been prescribed dipyridamole were interviewed, since this is first-line treatment locally. We ascertained if they were still taking dipyridamole, if they had experienced adverse effects and changes made to therapy as a result.

**Results:** Eighty-six patients were included; 50 (58%) were male. The initial stroke diagnoses were: TACS, eight patients (9%); PACS, 43 (50%); LACS, 16 (19%); POCS, 8 (9%); ICH, 4 (5%); and amaurosis fugax, 7 (8%). Antiplatelet or anticoagulant therapy had been prescribed to 83 patients (97%). Forty-six (55%) had been prescribed dipyridamole. Fourteen of these (30%) experienced adverse effects attributable to dipyridamole and 5 (11%) discontinued therapy. The most common adverse effects were headache [nine patients (20%)] and nausea/vomiting [three patients (7%)]. No aspect of medical history or concomitant medication
was associated with experiencing an adverse effect in patients prescribed dipyridamole on \( \chi^2 \) analyses.

**Conclusions:** Adverse effects are common following dipyridamole prescription and one-third of affected patients discontinue therapy. These findings are broadly compatible with results from RCTs including ESPRIT (20%) and ESPS-2 (25%).

**P69**

**NEW CLUES FOR GENTAMICIN-INDUCED NEPHROPATHY: INHIBITION OF GENE EXPRESSION OF LONG FATTY ACID AND CARNITINE TRANSPORTERS**

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This study investigated, on gene expression level, the mechanisms whereby gentamicin (GM) induces secondary carnitine deficiency and its relationship to GM-induced acute nephropathy in rats. The animals were divided into four treatment groups: Animals in group 1 were IP injected with normal saline for eight consecutive days. Rats in group 2 were injected with GM (80 mg/kg/day, I.P.) for eight consecutive days. Animals in group 3 were given L-carnitine (200 mg/kg/day, I.P.) for eight consecutive days. Animals in group 4 were received L-carnitine (200 mg/kg/day) 1 h prior to GM (80 mg/kg/day) for eight consecutive days. At the end of the treatment protocol, animals were sacrificed, kidneys were isolated and analyzed. GM resulted in a significant increase in the expression of tissue inhibitor of metalloproteinase-1 (TIMP-1), collagen type I and collagen type IV and a significant decrease in the expression of tissue inhibitor of metalloproteinase-1 (TIMP-1), collagen type I and collagen type IV and a significant decrease in the expression of organic cation/carnitine transporter (OCTN2), carnitine palmitoyltransferase I (CPT I) and matrix metalloproteinase-9 (MMP-9). Administration of L-carnitine to GM-treated rats resulted in complete reversal of GM-induced alteration in the expression of all studied genes to control values. Data from this study revealed that: GM induces carnitine deficiency by decreasing the expression of carnitine transporter, OCTN2, in the proximal tubules with the consequent increase in urinary carnitine excretion. GM induces its nephrotoxicity by increasing extracellular matrix accumulation secondary to increasing collagen synthesis and up-regulating TIMP-1 and down-regulating MMP-9 expression. Carnitine supplementation attenuates GM-induced nephropathy by modulating the relationship between MMP-9 and TIMP-1 which determines the integrity of the extracellular matrix.

**P70**

**PURPLE GASTRIC JUICE DISCOLORATION IN AN INFANT RECEIVING OMEPRAZOLE – INACTIVATION OF OMEPRAZOLE DUE TO PREMATURE COATING DISSOLUTION**

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**Introduction:** Omeprazole can be prescribed for gastro-oesophageal protection. It is usually administered in an oral pharmaceutical formulation preventing pH-dependent release of omeprazole before it reaches the duodenum.

**Methods:** We describe an infant who developed purple-colored gastric juice discoloration after buccal omeprazole administration. The mechanism of omeprazole discoloration is simulated in a laboratory setting.

**Results:** A 3-week old Caucasian boy developed symptoms of gastro-oesophageal reflux in combination with poor weight gain. Therefore, he received omeprazole 10 mg once daily in the evening. After opening the capsules (omeprazole 10 mg, Ratiopharm), the coated particles were inserted in the buccal space, immediately followed by breastfeeding. Five weeks after start of omeprazole therapy the boy’s refluxed gastric juice contained purple particles. This occurred almost every day, but only in the morning after sleeping. The omeprazole administration regimen was changed: after administration in the buccal space the child sucked a dummy teat for a few minutes, so that the particles were swallowed adequately. Consequently he was breastfed. The purple particles were then observed only twice in a couple of months.

In a laboratory setting addition of the coated particles to a basic solution resulted in degradation of the coating and a visible turbidity of the solution. By adding hydrochloric acid the solution was made acidic, turning the solution and particles yellow. Making the solution basic again, the yellow solution and particles turned violet-red. Addition of coated omeprazole particles to a neutral solution resulted in no visible coating degradation.

**Conclusions:** Buccal administration of coated omeprazole leads to premature dissolution and inactivation of omeprazole, visible as purple particles. Co-administration of a bit of an acidic (semi-) liquid product will decrease premature coating dissolution and reduce oral retention time because of facilitated swallowing. Grapefruit juice should be avoided because of an interaction with the hepatic cytochrome P450-3A4 enzyme. Furthermore, administration of omeprazole 30 min before feeding will optimize effectiveness, because the peak plasma concentration will coincide with mealtime.

**References:**


**P71**

**ADVERSE DRUG EVENTS IN INTRA-HOSPITAL TRANSFERS TO INTENSIVE CARE UNIT**

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**Introduction:** Adverse drug events (ADE) represent more than 6% of hospitalisations and can be life threatening. In a pilot study, we previously showed that ADE were implicated in 20% of intensive care unit (ICU) admissions. As 1/3 came from the hospital wards, we focused on assessing the contribution of ADE in intra-hospital transfers to ICU and to determine their preventability.

**Method:** Prospective observational study. Admissions to the ICU of the Geneva University Hospitals (36 beds) for 6 months were systematically analysed from January to July 2009. Demographic and medical data, drug history, clinical evolution and outcome were systematically collected. Clinical pharmacologists and ICU specialists decided independently on drug imputability, according to WHO criteria and preventability.

**Results:** From January to July 2009, 1310 ICU admissions were recorded, 323 of which were from the hospital wards. Most of the ADE were respiratory (30%), haemorrhagic (28%) or cardiovascular (20%) events. Accordingly drugs were mainly opioids, benzodiazepines or both, anticoagulants and beta-blockers. Clinical pharmacologists implied twice as much an ADE in ICU admission than the ICU specialist. More than one third of the AE were considered probably related with ICU transfer and 18% were considered preventable by both specialists.

**Conclusion:** These results confirm that ADE are frequently involved in ICU inward transfer and that one fifth are considered preventable. Our results stress the important contribution of the clinical pharmacist for improving intra hospital serious adverse event detection and underscore the need of developing strategies to try to prevent these ADE related transfers.
P72
PREVALENCE OF PREGABALIN IN URINE SAMPLES FROM PATIENTS TREATED FOR SUBSTANCE-DEPENDENCY
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Background: Pregabalin is a gamma-aminobutyric acid (GABA) ana-logue approved for the treatment of partial-onset epileptic seizures, neuropathic pain and generalized anxiety disorders. There has been increasing concern about the abuse potential of this drug. We therefore studied the prevalence of pregabalin in urine samples from patients treated for substance-dependency, and assessed whether its presence was based upon a prescription made by the respective patient’s physician.

Methods: Urine samples from 635 patients (69.1% males, mean age 37 years) treated for substance-dependency were analyzed for pregabalin in addition to a wide range of substances of abuse by using liquid-chromatography tandem mass-spectrometry (LC-MS/MS). Pregabalin-positive urine samples were linked to the presence or absence of a prescription for pregabalin for the time point the samples were taken and studied for the number of substances of abuse present in urine.

Results: Eighty-four patients (13.2%) had at least one positive urine sample for pregabalin. Thirty-two of these patients (38%) had no prescription for pregabalin by the time the positive sample was delivered. The mean number of other detected substances of abuse in urine was 2.54 for the group with pregabalin-positive urine samples vs. 1.70 for the group with pregabalin-negative urine samples (P < 0.001). In addition, the mean number of other substances of abuse present in urine was higher in those without vs. those who had a prescription (mean 3.25 vs. 2.1; P = 0.012).

Conclusion: Our data support the notion that pregabalin is likely to have an abuse potential that has to be taken into account in the choice of a drug regimen in patients treated for substance abuse.

Acknowledgements: This project was supported by a research grant financed by the Romanian Ministry of Education, Research and Innovation — PNII 12-102/2008.

P73
DETECTION AND CLINICAL CONSEQUENCES OF DRUG-DRUG INTERACTIONS IN HOSPITALIZED PATIENTS — PRELIMINARY RESULTS OF AN OBSERVATIONAL STUDY
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Introduction: The aim of this study was to assess the potential DDI’s as well as the DDI’s with clinical negative consequences (ADR’s) before and during hospitalization in patients admitted to an internal medicine ward.

Methods: The prospective study included, up to date, 108 patients admitted during 4 weeks in an internal medicine ward. The patients were interviewed at least twice during the hospitalization, regarding the drugs used and present symptomatology. Other information as medical history, medication used during the hospitalization, laboratory data and the results of other clinical investigation was collected from the patients’ charts. The potential DDI’s were identified using the Thomson Micromedex program. Each patient was monitored in order to detect the clinical consequences of DDI’s if present.

Results: Forty-two patients were enrolled in this study during 4 weeks. The mean age of patients was 59.60 years and the mean of hospitalization stay was 8.05 days. The average number of prescribed drugs per patient was 4.80 before admission and 6.36 for drugs prescribed during hospitalization. Seventyeight major potential DDI’s were identified. Seven DDI’s led to 9 ADR’s. Each patient was monitored in order to detect the clinical consequences of DDI’s if present.

Conclusion: Not all the potential DDI’s described in literature have clinical consequences. Of clinical importance are the ones that may lead to changes in the therapeutic effect of one of the two drugs involved in the DDI or to ADRs. When a high risk DDI is identified in a patient’s therapy, adequate monitoring of the patient is highly recommended in order to prevent an adverse outcome.
P76
ACTIVE MONITORING SYSTEM TO IDENTIFY AND CHARACTERIZE ADVERSE DRUG REACTIONS IN HOSPITALIZED PATIENTS IN ROMANIA

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Background: It is generally recognized that adverse drug reactions (ADRs) represent a constant concern of the health systems in terms of early recognition, proper management and prevention. Stimulated spontaneous reporting is a pragmatic method used for detection and characterization of ADRs in different hospital settings.

Objectives: Aiming to come with new data on ADRs, to study their nature and to target the most frequent ADRs in order to take future preventive measures, we implemented a stimulated spontaneous reporting program in two internal medicine departments from two secondary care academic teaching hospitals in Cluj-Napoca, Romania.

Methods: The stimulated spontaneous reporting program was initiated in 2009. All the physicians in the two departments are regularly reminded about the program, outlining the ADRs’ negative impact, and are asked to report all observed adverse events. The ADRs’ collection is paper based. A specially designed ADR form is filled out by the treating physicians when an adverse event is identified. The information is further evaluated for various parameters and then the data is entered into a database developed for data storage, data screening, data retrieval and future analysis.

Results: Two hundred adverse reactions were validated in 161 patients up to the end of 2010. The adverse events reported that were not validated were unlikely according to the causality assessment. The mean age of patients who experienced an ADR was 62 years. From the total number of patients with ADRs 72.05% were women. Patients with ADRs were hospitalized a total number of 1487 days, mean length of hospital stay being 9 (range 2–24). For 70 patients (43.5%), ADRs were the main reason for hospital admission. The overall incidence of serious ADRs was 70% out of the total ADRs reported. According to the MedDRA classification, the most frequent ADRs affected the gastrointestinal system, followed by metabolic, cutaneous, vascular and hepatic systems. The drugs most frequently involved were cardiovascular agents, anticoagulants, NSAIDs, antifungal and drugs acting on the nervous system.

Concerning the causality assessment, 70.74% ADRs were evaluated as being ‘probable’, 7.98% as being ‘possible’ and 7.45% were ‘definite’. Drug interactions were responsible for most of ADRs (19%), but we also identified other preventable causes. According to the selected preventability scale, 34% ADRs were classified as being ‘potentially preventable’ and 10.05% ‘definitely preventable’.

Conclusions: Active monitoring systems are of great value in a country where pharmacovigilance activities are at their very beginning, strengthening the ADRs reporting at the hospital level. Systematic detection and analysis of ADRs could help identifying common and repetitive patterns of preventable adverse events in hospitalized patients, with the final goal of preventing them.

P77
IMPROVING ADVERSE DRUG REACTION REPORTING THROUGH WORKSHOPS AND TELEPHONE EDUCATION: CLUSTER RANDOMIZED TRIAL AMONG PORTUGUESE PHYSICIANS

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Introduction: Adverse drug reactions (ADRs) are a significant public health problem, enlarged by the increased use of services in developed countries. Thus, post-marketed surveillance is fundamental for the discovery of such new ADRs. Spontaneous reporting of suspected ADRs by health professionals is fundamental to medical drug surveillance. However, the effectiveness of the system is limited by under-reporting. We conducted an extensive study purpose-designed to improve the effectiveness and relevance of spontaneous ADRs reporting among physicians in Northern Portugal. Educational intervention strategies were delineated to address this problem, performed by telephone interviews and workshops. Therefore, the present study aimed at evaluating the results of such educational intervention.

Methods: A cluster-randomized controlled trial was conducted with 6579 physicians working in Northern Portugal, in 2008. After randomization, 1481 physicians were placed into the intervention group (1056 in telephone interviews and 425 in workshops), while the control group was comprised of 5098 physicians. Statistical analysis was performed, based on the intention-to-treat principle, and generalized of linear mixed models were applied, using the penalized quasi-likelihood method.

Results: The intervention by workshops increased significantly (P ≤ 0.001) the rate of total spontaneous reporting of ADRs (RR = 3.971; 95% CI 3.863; 4.083), compared to the control group (RR = 0.924; 95% CI 0.923; 2.912). Conversely, after telephone intervention, no significant difference (P = 0.052) was observed in the reporting of ADRs (RR = 1.020; 95%CI 1.000; 1.041) compared with control group (RR = 1.724; 95% CI 1.076; 3.878). The effects of interventions in reporting rate of serious and high-causality ADRs indicated that the RR associated with workshops intervention for serious ADRs was 6.836 (95% CI 6.692; 6.983, P ≤ 0.001), and for high causality ADRs was 3.582 (95% CI 0.249; 2.841, P ≤ 0.001). The RR associated with telephone intervention for serious ADRs was 1.924 (95% CI 0.817; 4.530, P ≤ 0.001), and for high causality ADRs was 0.746 (95% CI 0.733; 0.759; P < 0.001).

Conclusion: The educational interventions by workshops increased significantly the quantity and relevance of spontaneous reporting of ADRs. Moreover, telephone intervention only increased the spontaneous reporting of ADRs in the first 4 months follow-up period. Accordingly, awareness programs could be proposed, every 4 months, to increase the effectiveness of those interventions.

P78
OBSTACLES AND SOLUTIONS IN SPONTANEOUS REPORTING OF ADVERSE DRUG REACTIONS

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Introduction: The spontaneous reporting system of adverse drug reactions (ADRs) is fundamental to drug safety surveillance but under-reporting is its major restriction. This bibliographic review sought to assess the influence of personal and professional characteristics on ADR reporting and to identify the obstacles and solutions associated with ADR reporting.

Materials and methods: A systematic review was conducted using the Pubmed, ISI Web of Knowledge and Google Scholar databases, including also search of cited papers. We included papers that were published in English and Portuguese, and covered a study population made up of health professionals (physicians, pharmacists and nurses).
Results: The review covered 25 papers that fulfilled the inclusion criteria. Some of the obstacles to spontaneous reporting considered were: ADRs diagnosis problem, problems related to time and resources, lack of information and access, procrastination, the organization of the pharmacovigilance system and problems related to potential conflicts. Solutions presented for improving spontaneous reporting were the resolution of institutional problems, like workload, teams’ organization and inducements, and also an improved cooperation and communication with the pharmacovigilance system leading to regular alerts and information about ADRs.

Conclusion: Studies like this are important to improve pharmacovigilance systems, having severe implications in public health. The identification of obstacles and solutions to spontaneous reporting can be seen as potentially modifiable factors in knowledge and attitudes of professionals.

Material and Methods: A retrospective observational study was conducted on the professional population of 49 pharmacies of Coimbra, a central Portuguese region, including 168 pharmacists and 64 pharmacy technicians. They were personally interviewed using a questionnaire that was adapted from previous studies (based on Inman’s ‘seven deadly sins’). They were surveyed about their knowledge and attitudes to ADR reporting and the factors that encourage and discourage ADR reporting.

Expected Results: The expected response rate is about 80–90% and the creation of the new form for reporting adverse drug reactions for healthcare professionals, in 2009, is also expected to have been a solution for increasing the rate of spontaneous reporting by pharmacists and pharmacy technicians.

P81
ADVERSE REACTIONS IN CRIMEA IN 2010
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In 2010 we analysed 1047 spontaneous reports about adverse reactions (ADRs) from 109 hospitals in Crimea. Most frequently ADRs were registered in first year children (82 cases) and in adults from 46 to 65 years old (282 cases). One lethal case caused by paracetamol was registered in pregnant woman. Seventy-seven patients were hospitalised. Most of drugs were used per os (547), others were prescribed intramuscularly (199), intravenously (213) or in topical forms (46 reports). Fifty-four patients had drug-associated allergic reactions in anamnesis. Only 306 cards informed about ADRs caused by monotherapy, in other cases patients got two or more medications. One hundred and seventy-nine patients got more than five drugs in the same time. Thousand and twenty-one patients had no long-term consequences after ADRs correction. It is interesting that some of cards reported about ADRs registered by doctors after weeks (53), months (21) and years (4) of therapy. Most of drugs were prescribed for patients with heart diseases (180). Five hundred and forty-three reports informed about skin rush, 119 about dyspeptic disorders, 81 about central nervous system symptoms, 49 about fever, 81 and 23 about angioneurotic oedema and anaphylactic shock. In 756 cases doctors prescribed additional medicines for correction of ADRs. We analysed all reports by pharmacological groups and found that in 335 (35%) cases ADRs were caused by antibacterial drugs, 115 (11%) reactions caused by ACE inhibitors and other drugs used in cardiology, 112 (11%) by NSAIDs and 111 (11%) by drugs influencing on metabolism, most of them by drugs with questionable efficacy. In antibacterial group of reports cephalosporins were prevalent (111 cases or 28% from group and 11% from total amount of cards) and ceftriaxone (48 cases) was ‘absolute leader drug’ not only in cephalosporins but in antibacterials and other groups too. Other ‘leaders’ are zidovudine (12 cases) in antivirals, paracetamol (26 reports) in NSAIDs, enalapril (14 cases) in ACE inhibitors, vitamins B combinations (19 cards) in metabolics. We performed detailed analysis of rationality of prescriptions and found medical and pharmaceutical errors, irrational combinations, off-label drug prescription, cases caused by ignoring of allergic anamnesis and contraindications from Instructions for medical use, irrational and unsafe correction of ADRs.
sionals (HCPs) of new drug safety concerns is questioned. Communication of safety-related regulatory action to prevent safety issues from occurring may be improved when more is known about HCPs' preferences. The objective of this study is to explore HCPs' preferences for improvement of communication of safety-related regulatory action comparing physicians' and pharmacists' views.

Methods: An anonymous, pilot-tested questionnaire was sent to a representative sample of Dutch physicians (n = 2396) and pharmacists (n = 1092) (Dec '09-Jan '10). Multiple choice and open questions were posed on perception of drug safety and DHPCs, familiarity with and action taken following DHPCs, satisfaction with DHPCs and preferences for alternative communication methods (both on a scale from 1 to 10). Descriptive statistics (χ²) were used to determine differences between HCPs.

Results: Twenty-seven percent (643) of physicians and 46% (507) of pharmacists responded. The majority of physicians (66%) and pharmacists (76%) considered drug safety information important for their profession (χ² P = 0.004). Remarkably, 19% of physicians and 11% of pharmacists had never seen or heard of DHPCs (χ² P < 0.001). Physicians indicated they undertook action following 24% (SD 22%), pharmacists following 34% (SD 25%) of the DHPCs issued. Physicians and pharmacists rated their satisfaction with DHPCs on average as 6.6 (SD 1.6) and 7.3 (SD 1.6) (χ² P < 0.001) respectively. HCPs preferred e-mail (7.6 SD 2.3), medical journals (7.5 SD 2.0) and electronic prescribing systems (7.1 SD 2.7) as alternative channels of drug safety information, preferably issued by regulatory authorities (8.1 SD 1.5) or own professional bodies (8.0 SD 1.7).

Conclusion: Although safety information of drugs is considered important, HCPs reported to undertake action in a minority of DHPCs issued. Pharmacists seem to be more involved with and responsive to safety issues than physicians. Satisfaction was comparable. Alternative methods should be developed using electronic systems and medical journals.

P83 IMPROVING PHARMACOVIGILANCE IN ONCOLOGY
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Background: In the last years, oncology has experienced a significant increase in new drug approvals: new target therapies may offer therapeutic benefit, but complete information regarding their toxicity depends on post-marketing vigilance. Most toxic classes of medicines tend to be associated to very few adverse drug reactions (ADR) reports. New biological substances increasingly require the use of registries in order to collect information on patients treated.

Materials and Methods: A pharmacovigilance project has been approved in Fatebenefratelli and Ophthalmic Hospital in 2009, with the aim to improve the attention to adverse events that may occur during cancer treatment and to facilitate a faster and better signal detection. The project is coordinated by the Oncology Department, with the collaboration of the Hospital Pharmacy. The ADRs detected by healthcare professionals are collected first in a specific database, and then in the Drug Regulatory Agency Pharmacovigilance System.

Results: Between March 2009 and December 2010, 116 ADRs in patients undergoing cancer treatment were identified: among these 27.60% were serious adverse events. In 32.76% of cases there was a delay of at least 7 days from the onset of rash using Naranjo criteria. In this 1-year prospective, observational study, carried out in Clinical Center Banja Luka, 34 adult patients with these ADRs were registered.

Discussion: Among the suspected drugs those with sulphonamide structure were the most frequent triggers for cutaneous ADR (sulphametoxyazol/trimetoprin, sulphalazine, furosemide), following by ACE inhibitors, lamotrigine, amino-penicillines, monoclonal antibodies. Found severe ADRs such as furosemide induced a red, blistering, peeling skin rash; DRESS syndrome induced by sulphalazine, exfoliative dermatitis induced by allopurinol, lamotrigine induced a diffuse erythematous, pruritic macular rash. After Dechallange ADR and symptomatic and supportive therapy all ADR were resolved slowly without consequences. Uncommon ADRs were ginvivitis induced by sulphametoxyazol/trimetoprin, which causal correlation were confirmed with Rechallenge, and itching induced by simvastatin. ACE inhibitors (mainly enalapril) were seen as possible causes in pruritis worsening. Other registered ADRs were common and mild or moderate.

Conclusion: Sulphonamides are the most common single drug associated with cutaneous ADR. Frequently prescribed drugs can induce serious and even potentially life threatening ADR.
PIGLITAZONE-ASSOCIATED BLADDER CANCER: THE CONTRIBUTION OF SPONTANEOUS REPORTING ANALYSIS ON THE BASIS OF FDA_AERS DATABASE

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Introduction: In September 2010, FDA announced the ongoing investigation on a possible association between long-term use of pioglitazone and bladder cancer. The aim of our study was to provide the contribution of spontaneous adverse event reporting analysis on causality assessment between bladder cancer and pioglitazone.

Methods: Association between antidiabetic drugs and bladder cancer was analyzed by the case/non reports recorded in FDA_AERS (Adverse Event Reporting System) between 2004 and 2009. Cases were represented by the reports of reactions included in the MedDRA high-level term 'Bladder neoplasms' for a given drug: non-cases were all the reports of other reactions associated to the same drug. For each drug, the association between such drug and bladder cancer was calculated by using the ADR reporting odds ratio (ROR). To weight the influence on ROR of male and older age, well known risk factors for bladder cancer, stratified analyses were performed. Furthermore, to consider the possible effect of notoriety bias a year-by-year analysis was performed.

Results: Overall, 93 reports of bladder cancer were retrieved, corresponding to 138 drug reaction pairs, with 31 concerning pioglitazone, 29 insulin, 25 metformin, 13 glimepiride, 8 exenatide; 22 others. ROR was significantly >1 for pioglitazone (4.30; 95% CI 2.82–6.52, P < 0.001), gliclazide (3.56; 1.42–8.39, P = 0.001) and acarbose (5.12; 1.61–14.33, P < 0.001), although for the last two drugs only a few cases were reported (6 and 4, respectively). Concerning pioglitazone, ROR resulted statistically significant both in female (5.19; 2.15–12.11) and male (3.86; 2.37–6.26), but only in older patients (>65 years); moreover the stratified year-by-year analysis showed a statistically significant ROR for 2004, 2006, 2007 and 2008.

Conclusions: We found a definite signal for bladder cancer associated with pioglitazone use. The demographic characteristics of the selected cases were consistent with bladder cancer epidemiology (male gender, old age). A weaker signal was also associated with gliclazide and a much weaker signal with acarbose. Of note, the occurrence of ≥5 events, although resulting in a statistically significant ROR, may be considered clinically meaningless because too much open to reporting biases. The presence of a signal for pioglitazone also before main relevant publications (PROactive 2005) supports the importance of this safety issue.

Materials and Methods: Data mining approach was carried out on publicly accessible FDA spontaneous reports (January 2001-December 2009) to retrieve cases where QT shortening/prolongation was reported.

Results: Among 1,644,133 total FDA reports, QT prolongation was reported in 3502 cases, whereas QT shortening only in 35 reports. Among cases reporting QT shortening, five drugs were reported twice as suspect (i.e. aripiprazole, atomoxetine, clozapine, moxifloxacin and ziprasidone). Digoxin and rufinamide (considered as QT shortening drugs), were reported in 3502 cases, whereas QT shortening only in 35 reports.

Conclusion: These reports are consistent with the hypothesis that QT shortening may predict arrhythmias, although the clinical impact of QT shortening remains elusive and further investigation is needed. Pharmacovigilance reports should meet a common standard of 'minimum requirements' to become a more reliable indicator of risk. The research leading to these results has received funding from the European Community’s Seventh Framework Programme (FP7/2007-2013) under grant agreement n° 241679 – the ARITMO project.
P91
REPORTING FREQUENCY OF QT-INTERVAL ABNORMALITIES IN THE FDA ADVERSE EVENT REPORTING SYSTEM (FDA_AERS)
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Introduction: QT prolongation is a recognized surrogate marker of proarrhythmia, namely Torsade de Pointes (TdP). Recently, interest has emerged on the possible arrhythmic risk associated with QT shortening.

Materials and Methods: From the publicly accessible FDA_AERS (January 2001-December 2009), spontaneous reports of TdP, QT prolongation/shortening were selected. The reporting frequency (restricted to drugs reported as suspect) was provided for top five agents by calculating the ratio between cases of interest and other reports in the database. The list was analyzed according to Arizona_CERT current information (www.azcert.org).

Results: From 1,644,133 total FDA reports, TdP was identified in 1482 cases (0.009%), QT prolongation in 3520 (0.002%), whereas QT shortening only in 35 reports (with 30 reported drugs). The reporting frequency for TdP was: cisapride (10.7%, 77 cases), sotalol (9.9%, 70), methadone (4.1%, 143), fluconazole (3.9%, 91), amiodarone (3.4%, 160). The rank for QT prolongation was: cisapride (38.7%, 223 cases), arsenic trioxide (13.5% 83), sotalol (10.7%, 75), nilotinib (10.6%, 94), methadone (4.3%, 150). All agents are grouped in the ‘Torsades List’ of the Arizona_CERT website, except fluconazole (‘Conditional Torsades List’). Rufinamide and digoxin (considered as QT shortening drugs) received only one report each.

Conclusion: The reporting frequencies of TdP and QT prolongation involved similar drugs and are in line with information reported by Arizona_CERT, although fluconazole deserves further investigation regarding its pro-arhythmic risk. The clinical impact of QT shortening remains elusive.

The research leading to these results has received funding from the European Community’s Seventh Framework Programme (FP7/2007-2013) under grant agreement n° 241679 – the ARITMO project.

P93
REPORTED INFORMATION CONTRIBUTING TO ADVERSE DRUG INTERACTION SAFETY SIGNALS
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Introduction: Many drug interaction safety signals from individual case safety reports (ICSRs) are based on well-documented, clinically strong index cases. This study was conducted to identify what information on the reports support a drug interaction safety signal and to what extent this information is registered in structured format.

Methods: Reports referred to in three published drug interaction signals were assessed using the Drug Interaction Probability Scale (DIPS) proposed by Horn et al. Reports in the WHO global ICSR Database, VigiBase and original files from respective country were reviewed. Altogether, 173 reports were analysed with a separate analysis of nine index cases. For each DIPS element, the number of times that it was available at all and in a structured format was measured. Information was presented separately for VigiBase and the original files.

Results: Particularly valuable information in the index cases were a reasonable time course, where the adverse reaction related to the regime of the affected (object) drug appeared in a reasonable time frame after the addition of the potentiating (precipitant) drug, and the resolution of the ADR upon withdrawal of the precipitant drug. For more than 50% of the index cases a plausible timeliness was provided in structured format on the original files (slightly less in VigiBase).

In general, structured data important for the causality of adverse drug interaction were more often provided in original files than in VigiBase.

Conclusions: Signals of suspected adverse drug interactions were often supported by suggestive time courses and resolution of the ADR upon dechallenge of the precipitant drug. This information is available in structured format on a fair proportion of reports and could potentially be used systematically.

P94
COMPARISON OF DRUG SAFETY SIGNAL DETECTION BETWEEN SPONTANEOUS REPORTING DATABASES AND EU-ADR LONGITUDINAL DATABASE NETWORK
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Introduction: In recent years, signal detection (SD) and data mining research has focused on leveraging longitudinal healthcare databases to detect signals. The EU-ADR project aims to exploit different European, longitudinal, electronic healthcare records databases for SD. The aim of this study is to compare SD results between FDA/WHO SRS databases and EU-ADR database network concerning six events (bulfus eruptions-BE, acute renal failure-ARF, acute myocardial infarction-AMI, anaphylactic shock-AS, rhabdomyolysis-RHABD, and upper gastrointestinal bleeding-UGIB).

Methods: The FDA and WHO SRS databases were mined from 1969-2010 for the MedDRA PTs of six events. Signal thresholds used were EB05 > 2 in presence of at least 1 report. Drugs that exceeded this threshold were identified as potential signals. EU-ADR platform consist of eight databases from four countries (Denmark, Italy, Netherlands, and United Kingdom), contributing data from 1996 - 2010. A custom-built software (Jerboa) elaborates locally harmonized input data and generates aggregated data which are subsequently analysed through different statistics (i.e.Longitudinal Gamma Poisson Shrinker). Drugs with statistically significantly (P < 0.05) increased posterior expectation of RR 2 have been identified as potential signals. Based on background incidence rate, for each event we estimated the required amount of exposure to test a drug as potential signal. Drugs not reaching this threshold were not included in the analyses.

Results: SRSs could explore, as potential signals, a broader number of drugs for the six events, in comparison to EU-ADR (range: 630–3393 vs. 87–856), particularly for those events commonly thought to be potentially drug-induced (i.e.BE: 2053-3393 vs. 228). On the contrary, EU-ADR may investigate a greater number of drugs concerning AMI (856 vs. 630–791). The highest proportion of signals detected in SRSs was for BE, ARF and AS, while for ARF and UGIB in EU-ADR.

Conclusions: EU-ADR longitudinal database network may complement traditional spontaneous reporting system for SD, especially for those adverse events that are frequent in general population and are not commonly thought to be drug-induced.

P95
A COMPARISON OF MEDICATION SURVEILLANCE BETWEEN NON-ONCOLOGIC AND ONCOLOGIC MEDICATION
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Introduction: Drug interactions in oncology are of particular importance owing to the narrow therapeutic index and the inherent toxicity of anticancer agents. The aim is to inventories the current situation referring to oncolytic medication surveillance and make a comparison between non-oncolytic (NOM) and oncolytic medication (OM).
Methods: A questionnaire was sent to hospital pharmacists (HP). The relative and absolute percentages for the different questions were evaluated.

Results: HP check routinely: kidney function (81%), all blood values (22%), blood cell count (16%), electrolytes (13%), INR (6%) and blood oncolytic levels (6%). In 41% of the cases HP have access to patients’ home medication. Information concerning OM is communicated to general practitioner in 40% of the cases, to community pharmacies in 20% and not communicated at all in 40%.

Conclusion: Knowing the OM high toxicity and narrow therapeutic range, there is still much work to do to achieve the same medication surveillance level and quality as currently existing for NOM. From the 80, 58 (73%) HP responded (see Table 1).

<table>
<thead>
<tr>
<th>Table 1. Comparison NOM and OM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prescribing system</td>
</tr>
<tr>
<td>NOM</td>
</tr>
<tr>
<td>38% Electronic</td>
</tr>
<tr>
<td>15% Manual</td>
</tr>
<tr>
<td>47% Combination</td>
</tr>
<tr>
<td>Person inserting the medication in the electronic system</td>
</tr>
<tr>
<td>NOM</td>
</tr>
<tr>
<td>32% P and nurse/pharmacy technician</td>
</tr>
<tr>
<td>Performing way for interaction’s medication surveillance</td>
</tr>
<tr>
<td>NOM</td>
</tr>
<tr>
<td>15% Manual</td>
</tr>
<tr>
<td>22% Combination</td>
</tr>
<tr>
<td>Person performing the medication surveillance</td>
</tr>
<tr>
<td>NOM</td>
</tr>
<tr>
<td>3% P</td>
</tr>
<tr>
<td>45% combination</td>
</tr>
<tr>
<td>OM</td>
</tr>
<tr>
<td>17% Electronic</td>
</tr>
<tr>
<td>54% Manual</td>
</tr>
<tr>
<td>29% Combination</td>
</tr>
<tr>
<td>71% Oncologist</td>
</tr>
<tr>
<td>14% Pharmacist</td>
</tr>
<tr>
<td>12% Automatic</td>
</tr>
<tr>
<td>41% Manual</td>
</tr>
<tr>
<td>31% no surveillance</td>
</tr>
<tr>
<td>29% HP</td>
</tr>
<tr>
<td>26% P</td>
</tr>
<tr>
<td>16% combination</td>
</tr>
</tbody>
</table>

P96 MEDICATION SURVEILLANCE ON IV ONCOLYRICS: EXTRA SERVICE OR A NECESSITY?

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Introduction: Medication surveillance is not commonly performed for oncolytic drugs (1–3). The objective of this study is to determine the prevalence and relevance of interactions with oncolytics in ambulant cancer patients receiving one or more intravenous oncolytic gifts.

Methods: All ambulatory cancer patients receiving one or more intravenous oncolytic gifts during the period October 2008 to April 2010 were included. Oncolytic drug schemes were obtained from the electronic prescribing system and medication history was obtained from the electronic prescribing system (EPS). Medication and oncolytics were entered into the EPS and medication surveillance was performed retrospectively using the G-standard (inclusive level of evidence and relevance). Interactions were clinically relevant, when one drug can affect the metabolism of another drug causing a severe change in concentration.

Results: A total of 527 ambulatory cancer patients was enrolled. The mean age of the patients was 63.6 years and 303 were female (55%). In 24 patients a total of 58 oncolytic drug-related interactions was detected of whom five were clinically relevant. These interactions are shown in table 1.

Conclusion: Knowing the OM high toxicity and narrow therapeutic range, there is still much work to do to achieve the same medication surveillance level and quality as currently existing for NOM. From the 80, 58 (73%) HP responded (see Table 1).

Table 1. The oncolytic drug-related interactions (*are relevant)

<table>
<thead>
<tr>
<th>Interfering drug</th>
<th>No. (%)</th>
<th>Evidence</th>
<th>Relevance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acenocoumarol</td>
<td>46 (79)</td>
<td>1</td>
<td>B</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>1 (1.8)</td>
<td>2</td>
<td>B</td>
</tr>
<tr>
<td>Phenytion</td>
<td>3 (5.2)</td>
<td>3</td>
<td>D</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>1 (1.8)</td>
<td>2</td>
<td>D</td>
</tr>
<tr>
<td>Valproic acid</td>
<td>1 (1.8)</td>
<td>2</td>
<td>D</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>2 (3.5)*</td>
<td>1</td>
<td>D</td>
</tr>
<tr>
<td>Folic acid</td>
<td>2 (3.5)</td>
<td>1</td>
<td>D</td>
</tr>
</tbody>
</table>

P97 NIFEDIPINE INDUCED THROMBOCYTOPENIA. A CASE REPORT

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Nifedipine is one of the most frequently prescribed calcium channel blocker in Romania, especially in the elderly hypertensive population. Nifedipine induced thrombocytopenia is a rare adverse reaction. We want to describe a case of an 80-year-old woman known with arterial hypertension and mild renal failure, following treatment with nifedipine 20 mg daily for more than a year, who was admitted to hospital with dyspnea, fatigability, myalgia, arthralgia. Laboratory investigations showed on admission day a platelet count of 64000. After excluding other causes for thrombocytopenia and after the discontinuation of nifedipine was followed by a raise in the platelet count to 141,000 after 6 days, a diagnosis of drug-induced thrombocytopenia was considered.

The incidence of thrombocytopenia with nifedipine based upon uncontrolled experiences is >0.5%. The onset of nifedipine induced thrombocytopenia can range from several days to several months since the beginning of therapy. Clinical signs are obvious only at platelet counts below 50,000. The reaction is immune mediated. Although in this case the adverse reaction was not severe and it was reversible at discontinuation of the drug, thrombocytopenia is a serious condition which can endanger a patient’s life, especially in the elderly. In this particular case, nifedipine induced thrombocytopenia prolonged the patient’s hospitaliza-
tion. According to the Karch-Lasagna probability scale, we evaluated this adverse reaction as being probable.

P98
ARE USERS OF ‘LEGAL HIGHS’ (NOVEL PSYCHOACTIVE SUBSTANCES) AT RISK OF ACUTE CAFFEINE TOXICITY?
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Introduction: There is increasing recreational use of novel psychoactive substances (often known ‘legal highs’) across Europe. Typically these substances are purchased from the Internet and there is often limited information available to users on the contents of the product and/or the potential adverse effects associated with their use. We report the results of analysis of novel psychoactive substances that were found to contain high concentrations of caffeine, which have the potential for significant caffeine-related toxicity.

Methods: Seven different novel psychoactive substances were purchased from different Internet suppliers that would dispatch them to a UK-based postal address. None of the products purchased stated on the website that they contained caffeine. Upon receipt, the products were weighed and the contents were analysed by gas-chromatography mass-spectrometry (GC-MS).

Results: All seven products ordered were dispatched and received; all weighed approximately 1 g on receipt. Analysis by gas-chromatography with mass-spectrometry (GC-MS) demonstrated that all seven products contained only caffeine; no other psychoactive substances (pharmaceutical, novel psychoactive substances or controlled drugs) were detected.

Discussion: These novel psychoactive substances contained approximately 1 g of caffeine per product. The caffeine content of commonly purchased caffeine-containing products is up to 100 mg/product (e.g. cola 45 mg/can, Red Bull 80 mg/can, coffee 40–100 mg/cup). Excessive consumption of these products is associated with the development of acute caffeine toxicity. There is the potential for significant acute caffeine toxicity in individuals using novel psychoactive substances containing high concentrations of caffeine, particularly as users will often taken 1 g or more in a single use session. Clinicians need to be aware of this potential, and ensure that management of these individuals is targeted appropriately.

P99
DETECTION OF THE PRECURSOR BENZOPHENONE IN INDIVIDUALS WHO HAVE USED LEGAL HIGHS CONTAINING DIPHENYL-2-PYRROLIDINEMETHANOL (D2PM)
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Introduction: Previous reports have suggested that legal highs (novel psychoactive substances) sold over the internet are of high purity and the detection of precursors in final products is unusual. We describe two cases of acute sympathomimetic drug toxicity in individuals using novel psychoactive substances containing high concentrations of caffeine, particularly as users will often take 1 g or more in a single use session. Clinicians need to be aware of this potential, and ensure that management of these individuals is targeted appropriately.

Cases: Case 1: 33 year old male ingested eight capsules of the legal high ‘Benzofury’ over the course of an evening. Initially, he had a ‘high’ similar to that he experienced with mephedrone (4-methylmethcathinone). He presented to the Emergency Department (ED) about 56 h post-ingestion with ongoing symptoms of anxiety/agitation and prolonged insomnia. On examination there were no features of acute sympathomimetic drug toxicity (HR 71 bpm, BP 140/95 mmHg, Temp 36.0°C, neurological examination normal).

Case 2: 29 year old male nasally insufflated approximately 1 g of a legal high that he purchased as ‘NRG-3’ and orally ingested a small amount of ‘MDMA’ powder. He developed intermittent visual hallucinations together with prolonged and ongoing anxiety, agitation and insomnia; he had taken an over-the-counter product containing diphenhydramine to try and induce sleep. He presented to the ED 72 h post-ingestion due to the prolonged nature of his symptoms. On examination there were no features of acute sympathomimetic drug toxicity (HR 80 bpm, BP 138/82 mmHg, Temp 36.5°C, neurological examination normal).

Urine (Case 1 and 2) and serum (Case 1) were collected and analysed by gas-chromatography mass-spectrometry (GC-MS). Both patients were reassured that their symptoms would resolve and were discharged home.

Results: Case 1: Urine positive for benzophenone and D2PM; serum D2PM concentration 0.22 mg/l
Case 2: Urine positive for MDMA and MDMA metabolites, mephedrone and mephedrone metabolites, diphenhydramine, D2PM and benzophenone.

Discussion: These are the first reports of the detection of the benzophenone precursor in biological samples taken from individuals who have used D2PM. The pipradrols, such as D2PM, were thought to be commercially produced from the precursor diphenylethanolitrile. The detection of benzophenone in these patients suggests that the D2PM used was less likely to have been commercially produced. Detailed analytical screening of individuals presenting with acute toxicity after use of legal highs can potentially provide information on the synthetic routes being used for these drugs, which can inform legislative authorities to ensure that appropriate monitoring and control of the relevant precursors is in place.

P100
A SUBJECT MANAGEMENT SYSTEM FOR SAFETY AND RELIABILITY IN THE EARLY CLINICAL TRIALS IN JAPAN BY JACIC

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Introduction: In the early clinical trials, safety of subjects as well as integrity of research findings is the primary interests of both investigators and sponsors. In 1989, JACIC was organized by Japanese clinical pharmacology units, where most of the early clinical trials in Japan are performed. In 1991, JACIC organized “Registration center for medical volunteers” and activated the management system to ensure safety and reliability by prohibiting subjects from taking part in two or more clinical trials by confirming required waiting period without interfering the subject’s willingness.

Methods: The system provides member institutes of JACIC with the status of the specific screened subject about his/her history of attending clinical trials. Under the system, the subjects are cross-checked through the database held at the center.

Results: The registration center has been utilizing the system since 1992 and over 480 thousand subjects were cross-checked and around 1000 subjects are identified as violators and are eliminated every year before entering clinical trials. Several cases as violators failed to be eliminated were found by cross-checking them when a new member institute joined in JACIC.

Conclusion: JACIC convince that the system well contributes to protecting such double-registrations, however, understand that the system is only applicable within member institutes and healthy subjects. The system should be employed to non-member institutes in order to boost up its coverage for healthy and patient volunteers to be participated in PK/ PD studies.
P101 PATTERN OF INTOXICATIONS IN THE EMERGENCY SERVICE AT MADEIRA ISLAND HOSPITAL

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Introduction: Intoxication is a significant and worsening public health problem in many countries and one of the most common reasons for visiting emergency services. Most intoxications result from industrial and technological development, that provides thousands of toxic substances. In general pharmaceuticals, pesticides, drug abuse and industrial chemicals are recognized as intoxicating agents. Studies of epidemiological aspects of poisoning cases are essential for designing strategies and preventive measures to reduce the impact of this problem. The objective of this observational retrospective study is to analyze all poisoning admitted in the emergency service at Madeira Island hospital, occurred between January and December 2009.

Methods: It was conducted a review study to investigate the incidence of poisoning in order to compare the etiological and demographical characteristics of poisoning patients. The data collected in the hospital will be analyzed in two groups: Group 1 - related to the patient: gender, age, occupation and place of origin (urban or rural), Group 2 - related to intoxication: The cause of poisoning (accidental, occupational, suicidal and intentional), route of exposure to the causative agent of intoxication, frequent symptoms, identification and classification of substances involved in poisoning episode. The data will be performed using the SPSS software.

Expected Results: Through this retrospective analysis, it is expected to confirm some of the information acquired in scientific articles related to intoxications and establish connections with the data collected. Let fonet icamente

P102 PATTERN OF INTOXICATIONS IN THE EMERGENCY SERVICE AT S. JOÃO HOSPITAL IN OPORTO

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Introduction: The World Health Organisation refers to poisoning, which is an important public health problem and a frequent cause of admission into hospitals. There are many chemicals responsible for the poisoning; some of them are substances that we contact in our day-to-day. The aim to investigate the demographic and etiological characteristics and toxic agents of intoxication cases in adults admitted on the S. João Hospital in Oporto, Portugal, from January to December 2009.

Methods: This retrospective observational study will include all intoxication admissions at the S. João Hospital emergency during the year 2009 through data collection from medical records of patients. The data will be organized in tables and these tables will contain the study variables and hypotheses. The hypotheses will be classified as the various codes in order to facilitate analysis. The SPSS version 17.0 will be used to analyse the data.

Expected Results: In a total of admissions at the S. João Hospital during the year of 2009, we hope to purchase data, which lead us to conclusions about the brand of intoxications, etiology, and possible treatment. The study may provide a tool for promotion of public health, prevention and safe use of medicines.
METABOLIC SYNDROMES: DIABETES MELLITUS

PI04
EFFECTS OF ANTI-DIABETIC DRUG PHENSUCCINAL ON METABOLIC PROCESSES AND IMMUNE SYSTEM WITHIN THE METABOLIC SYNDROME

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Metabolic syndrome (MS) comprises a complex of the hormonal and metabolic disorders which increase the risk of cardiovascular pathology, especially in postmenopausal women. The fast prevalence of this syndrome defines the significance of exploration of its effective and safe drug correctors. The paper studies effects of the original anti-diabetic drug from the succinates group, Phensuccinal (PhS), on the metabolic and immune status within the insulin resistance syndrome model with hypogestrogenia. The postmenopausal MS model was simulated by a 5-week introduction of 30% saccharose to ovarioectomized rats. PhS was brought in per os dosaged 50 mg/kg/5 weeks. We studied the state of carbohydrate, lipid and protein metabolism, of oxidative protein modification (OPM), and of cellular and humoral immunity units. We found that PhS did not affect the level of basal glycemia but restored the carbohydrate tolerance (increased the insulin sensitivity and glucose utilization levels). PhS proved not to change the fructosamine level, i.e., not to decrease intensity of the primary reactions of nonenzymatic glycosylation, nor affect the lipid and protein metabolism. On the other hand, PhS application under the MS conditions may activate the OPM processes (increase of 2,4-dinitrophenylhydrazones content) in the blood serum. Within the immune protection system, which under the MS is characterized by the depression of the humoral and disbalance in the phagocytic & T-cellular units, PhS introduction proved to normalize the indices of phagocytic and metabolic activity of neutrophils, increase the level of antibodies to 17β-estradiol in blood serum and the number of lymphocytes in rosetting reaction with the insulin load. We concluded that PhS application under the MS conditions fosters restoration of the carbohydrate tolerance and promotes normalization of the phagocytic and stimulation of the T-cellular immunity units but can intensify the OPM processes.

PI05
PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR γ POLYMORPHISM IN IRANIAN POPULATION: RELATION WITH INSULIN RESISTANCE AND RESPONSE TO PIOGLITAZONE IN DIABETES TYPE 2

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Introduction: The peroxisome proliferator-activated receptor γ (PPARγ) has important effects on insulin sensitivity, obesity and diabetes. Pioglitazone improves insulin sensitivity by activating PPARγ. In view of strong evidence of interindividual variability in therapeutic response to pioglitazone, this study was designed to search for an association between type 2 diabetes mellitus (T2D) and single-nucleotide polymorphisms (SNPs) in PPARγ (Pro12Ala) and to investigate whether these genetic variants affect pioglitazone response in a population of Iranians with diabetes.

Methods: A total of 101 patients with T2D were treated for 12 weeks with pioglitazone (15 mg/day). Paraclinical parameters were measured before and after therapy. We genotyped 128 control participants and all patients with T2D. The Pro12Ala polymorphism in PPARγ was detected with real-time PCR.

Results: The Ala allele was found in 7% of the control participants vs. Three percent of those with T2D (P = 0.036). The genotypic frequencies of Pro/Ala were 5.94% in the former group vs. 14.06% in the latter (P = 0.04). There were significant changes in some laboratory values and biochemical markers of insulin sensitivity after pioglitazone therapy. The Pro12Ala polymorphism was associated with significant changes in insulin/glucose ratio after treatment (P = 0.015 in Pro/Pro and P = 0.005 in Pro/Ala genotypes).

Conclusions: Our findings suggest that in carriers of the 12Ala variant, pioglitazone significantly reduced the risk of type 2 diabetes, and in diabetic patients with the Pro12Ala genotype, the therapeutic response to treatment was better than in patients with the Pro12Pro genotype, although the difference between groups did not reach statistical significance.

Keywords: Iranian Population, Peroxisome proliferator-activated receptor γ, Polymorphism.

PI06
THE ASSOCIATION OF ADIPONECTIN (45 T/G), ADIPONECTIN RECEPTOR 2 (+795 G/A) POLYMORPHISMS WITH TYPE 2 DIABETES AND PIOGLITAZONE RESPONSES IN IRANIAN POPULATION

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Introduction: Thiazolidinediones (TZD) represent a model of pharmacogenetics. They are able to increase adiponectin level and cause insulin sensitivity. adiponectin a novel adipose-derived plasma protein is low in patients with obesity and type 2 diabetes (T2D). Adiponectin, Adiponectin receptors gene polymorphisms have been studied recently for their effects on obesity and diabetes. Our aim is to study any association between Type 2 diabetes mellitus and two single nucleotide polymorphisms (SNPs) in the adiponectin gene (T/C45), adiponectin receptor2 gene (795G/A) and investigating if these genetic variants affect pioglitazone response in Iranian diabetic population.

Methods: We genotyped 128 non-diabetic controls and 101 patients with diabetes type 2 for SNPs T45G and 795G/A of adiponectin and adiponectin receptor 2 gene by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Patients were treated with pioglitazone (15 mg/day) for 12 weeks. Paraclinical parameters were measured before and after treatment.

Results: There is a significant differences in biochemical markers before and after pioglitazone therapy. In 795G/A there is significant differences in Fasting Blood Sugar between different genotypes after pioglitazone treatment (P = 0.009). There is also a significant differences in mean change of I/G ratio before and after therapy in different genotypes of 45T/G (P = 0.035). There is no statistically significant differences in allele frequencies of SNPs 45 and 795 comparing control with Type 2 diabetic subjects. T frequency 87.11% vs. 81.68%, P = 0.071 for SNP45 and G frequency 76.17% vs. 80.20%, P = 0.179 for SNP795G/A in patients and control group respectively. But there is significant differences in genotypic frequency of 45T/G between patients and control group (P = 0.032).

Conclusion: Our findings suggest that 45 T/G of the adiponectin gene may be the important determinants for T2D in Iranian patients. However, adiponectin allele 45T/G and adiponectin receptor2 allele 795G/A polymorphisms are not significantly associated with the therapeutic efficacy of pioglitazone in Iranian patients with T2D.
Keywords: Adiponectin gene, adiponectin receptor-2 gene, Iran, polymorphisms, population.

**P107**

**INTERACTION OF SUBCUTANEOUS LIXISENATIDE 20 MG OD ON PHARMACOKINETIC AND PHARMACODYNAMIC PROPERTIES OF ORAL RAMIPRIL 5 MG QD IN**

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**Introduction:** Lixisenatide is a GLP-1 agonist in development for once-daily SC administration in patients with T2DM. T2DM patients frequently receive a concomitant ACE inhibitor. We assessed the effect of multiple doses of lixisenatide on steady-state PK and PD properties of ramipril and its active metabolite ramiprilat in an open, randomized, 2×2 cross-over study.

**Methods:** Healthy young males and females (n = 30) were randomized to one of two treatment sequences to receive 5 mg ramipril alone in one period and 5 mg ramipril plus 20 μg lixisenatide for 6 days in the other. Twenty microgram lixisenatide treatment was preceded by a 7-day run-in with 10 μg lixisenatide. Ramipril treatments were separated by 23-day washout periods. Twenty four hour PK profiles of ramipril and ramiprilat were taken on Day 6 of ramipril dosing in both periods. PD response to ramipril was evaluated on the change from period baseline in AUC0-24h in each period for the tetrapeptide N-Acetyl-Ser-Asp-Lys-Pro (AcSDKP) after 6 daily doses of 5 mg ramipril alone and under coadministration with lixisenatide.

**Results:** Lixisenatide was well tolerated when given alone and in combination with ramipril. One subject developed an allergic reaction of moderate intensity on Day 2 of ramipril + lixisenatide coadministration, with ubiquitous urticarial exanthema. The allergic reaction resolved without treatment, and the subject was withdrawn and excluded from PK/PD analyses. The 90%CIs of the ratios for Cmax and AUC0-24h of ramiprilat plasma concentrations in both periods were within the protocol-specified boundaries [0.80–1.25], demonstrating lack of interaction on the PK of ramiprilat at steady state. For ramipril PK, lack of interaction was not demonstrated: the 90%CI of the ratio for AUC0-24h was not entirely within the reference interval and it was completely outside the range for the secondary parameter Cmax. Mean change from period baseline in AUC0-24h of AcSDKP was higher under coadministration of ramipril + lixisenatide vs. ramipril alone, with a mean ratio of 1.19 and a 90%CI of 1.03–1.37, thus not being entirely within the reference interval [0.80–1.25].

**Conclusions:** There was no interaction of a multiple dosing with lixisenatide on the PK of ramiprilat, the active metabolite of ramipril, whereas lack of interaction on the PD parameter of ramiprilat cannot be concluded. However, with a ratio of 1.19, the overall effect is not considered to be a clinically relevant interaction.

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**P108**

**EFFECTS OF DIFFERENT DOSES PREPARATION OF ZINC ON GLYCHEMA AND ANTIOXIDANT DEFENSE SYSTEM IN EXPERIMENTALLY INDUCED DIABETIC RABBITS**

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**Introduction:** Researches with micronutrients are getting more and more important in science and also in practice. In this view zinc, copper and selenium are having a special role in preventing micro- and macrovascular diabetic complications, as integral components of antioxidant enzymes and also as co-factor of enzymes and hormones involved in the metabolism of glucose and lipid. The aim of this study was to evaluate the effects different doses preparation of zinc on glycemia and parameters of antioxidative defense: superoxide dismutase (SOD) and total antioxidant status (TAS) in alloxan-induced diabetic rabbits.

**Methods:** The study was conducted on fourteen New Zealand rabbits, weighing 2–3.5 kg. Experimental diabetes was induced in rabbits by intravenous injection of alloxan (80 mg/kg BW). Three weeks after induced experimental diabetes, rabbits were treated orally appropriate doses of chelated zinc: first dose 15 mg, after the washout period (10 t1/2), second dose 25 mg, after the washout period (10 t1/2), third dose 50 mg. Blood samples were taken at certain time intervals: before alloxan induced diabetes, after alloxan induced diabetes, after the first dose of zinc, after the second dose of zinc, after the third dose of zinc.

**Results:** The preparation of zinc did not cause a statistically significant reduction in serum glucose level when administered in single doses (15, 25 and 50 mg). The activity of SOD was significantly increased after the single dose preparation of zinc at a dose 15 mg (P < 0.05), 25 mg (P < 0.001) and 50 mg (P < 0.001) in relation to the activity recorded before the application of zinc. Also, after the application first, second, and third dose of zinc in diabetic rabbits was recorded statistically significant increase of TAS in relation to the value recorded before the application of zinc (P < 0.001). Glucose concentrations negatively correlated with superoxide dismutase activity.

**Conclusions:** This indicates that oral application preparation of zinc can reduce the harmful effects of oxidative stress in diabetes.

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**P109**

**PROTECTIVE EFFECTS OF COMBINED THERAPY OF GLICLAZIDE WITH CURCUMIN IN EXPERIMENTAL DIABETIC NEUROPATHY IN RATS**

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Diabetic neuropathy is the most common chronic complication of diabetes. Aim: The aim was to evaluate the protective effects of curcumin against neuropathy in gliclazide treated diabetic rats.

**Methods:** Diabetes was induced by intraperitoneal injection of streptozotocin (45 mg/kg, dissolved in cold citrate buffer adjusted to pH 4.5). Diabetic animals were given gliclazide (GZ; 10 mg/kg, p.o.) or combined with curcumin (CM; 100 mg/kg, p.o) or gabapentin (GBP; 30 mg/kg, i.p. as positive control). Behavioural response to thermal (hot plate and tail flick), mechanical (tail pinch) pain and some biochemical tests (serum glucose, c-peptide, peroxynitrite, lipid peroxides and tumor necrosis factor alpha) were measured after five consecutive weeks of daily treatment.

**Results:** The combined treatment of CM with GZ significantly increased hot plate latency time [12.63 ± 1.27 s vs. 4.883 ± 0.28 s in diabetic control group (DC)]. Moreover, the combination significantly increased tail flick latency time (13.31 ± 1.14 s vs. 7.23 ± 0.49 s in DC and the threshold of mechanical hyperalgesia was significantly elevated. The serum glucose and c-peptide levels were significantly improved in the combined treatment compared to DC. Lipid peroxide and peroxynitrite production were significantly lowered in serum (2.53 ± 0.05 vs. 3.59 ± 0.09 nmol/ml malondialdehyde in DC at P < 0.05) and [61.40 ± 10.80 vs. 130 ± 10.74 umol/ml total serum nitrate/nitrite in DC at P < 0.05]. Serum TNF alpha was also reduced in the combined treatment to 96.36 ± 3.53 vs. 277.20 ± 20.03 pg/ml in DC (P < 0.05).

**Conclusion:** Data suggest that the combination of curcumin with a conventional antihyperglycemic may protect against the development of diabetic neuropathy.
P110

RAS SIGNALING MEDIATES GLUCOSE STIMULATED ERYTHROPOIETIN SYNTHESIS IN CULTURED HUMAN RETINAL PIGMENT EPITHELIAL CELLS

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Introduction: Human retinal pigment epithelial cells (hRPE) is implicated in the pathogenesis of proliferative diabetic retinopathy. Erythropoietin (Epo) is an angiogenic factor which is synthesized in hRPE cells. Since very little is known about the effect of elevated glucose on Epo production and its mechanism, we investigated the effect of elevated glucose on Epo synthesis in cultured hRPE cells.

Methods: Primary hRPE cell cultures were exposed to glucose (0–20 mM). Cell viability was assessed by the trypan blue exclusion method (T) and cell proliferation was measured by 3H-thymidine incorporation (3H-thy). Morphological analysis was performed by phase contrast microscope. 14C-methionine labeled Epo (14C-Epo) synthesis was determined by immunoprecipitation using anti-Epo. To study the role of RAS protein in Epo synthesis, we used RAS inhibitor, mevastatin. Statistical significance was established by Student’s t-test.

Results: FBS stimulates hRPE cell proliferation in a dose-dependent manner as measured by 3H-thy and T. Elevated glucose had no effect on hRPE proliferation. Morphological analysis showed that hRPE cells grew normally as a monolayer and did not show any abnormalities in presence of glucose. But, elevated glucose stimulated 14C-Epo in hRPE cells in a dose-dependent manner. This stimulation in Epo synthesis was inhibited by mevastatin (30 μM) (4204.63 ± 1017.18 vs 7371.79 ± 1775.40, mean ± SEM, n = 7, P < 0.0008).

Conclusions: Glucose does not stimulate hRPE cell proliferation but increases Epo production in cultured hRPE. In addition, the RAS kinase pathway may mediate this glucose-stimulated Epo production. These data suggest that a RAS kinase pathway inhibitor may be of therapeutic value in treating proliferative diabetic retinopathy.

Supported by a Skillman Foundation Endowment.

P111

EXPRESSION OF GENETIC HORMONES RECEPTORS IN THE TESSTITLES OF YOUNG AND ELDER DIABETIC MICE

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Introduction: Experimental diabetes in animals induces marked alterations of gonadal androgenic functions: morphological alterations of testicular cells. The aim of this preliminary study was to investigate the expression of testosterone, estrogen and progesterone receptors in the testicles of young and elder diabetic mice.

Materials and methods: Twelve Balbc mice were used, 6 of 4 weeks age and 6 of 16 weeks age. Each age group was further divided in two subgroups. The first subgroup included two indexes that received only saline water. In the second one, alloxane was administered, so that the mice became diabetic. During the experiment the diabetic mice received insulin for keeping the levels of blood glucose between 250–300 mg/dl. After 5 weeks, the testicles were excrated for immunohistochemical evaluation with genetic hormone receptors antibodies.

Results: The capillaries, the interstitial tissue and the Leydig cells showed mild immunostaining for progesterone and estrogen receptors in the young aged group. The same was also noticed for the androgen receptors in the Leydig cells. Nevertheless the immunostaining of the capillaries and the interstitial tissue was significant. In the elder group, there was a weak response for all hormones? receptors. However, when stained with progesterone and testosterone receptors antibodies, the results were assessable for the capillaries.

Conclusion: A possible role of estrogen in male reproductive function is suggested but without revealing a direct cause-effect relationship. A reduction in the basal testicular content of progesterone testosterone is noticed in alloxane-diabetic rats.

P112

EFFICACY OF ANTIPLATELET THERAPY WITH ASPIRIN IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

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Introduction: The aim of the study was to determine the efficacy of antiplatelet therapy in patients with type 2 diabetes mellitus while taking into account the possible effects of comorbidities of the patients treated, pharmacotherapy employed, and laboratory parameters monitored.

Materials and methods: In 101 patients with type 2 diabetes mellitus on antiplatelet therapy with 100 mg aspirin daily, we examined the urinary levels of 11-dehydrothromboxane B2. The findings were compared with those obtained from a control group of age- and sex-matched 101 non-diabetic patients. In both groups, we evaluated the clinical and laboratory parameters monitored and the medicines co-administered.

Results: A higher incidence of ineffective antiplatelet therapy was found in the group of patients with diabetes compared with the control group. Elevated urinary levels of 11-dehydrothromboxane B2 were found in 46.5% of diabetic patients and in 30.7% of controls (P = 0.02). In the laboratory parameters monitored, there were statistically significant differences between the two groups in values of glycemia and C-reactive protein levels only. By contrast, no effect of comorbidities, other laboratory parameters monitored, and concomitant medication was demonstrated.

Conclusions: Antiplatelet therapy with aspirin in patients with type 2 diabetes is less effective compared with individuals without diabetes. No role of comorbidities or concomitant medication in the higher incidence of antiplatelet therapy has been shown. Diabetes-related metabolic changes per se decrease the efficacy of antiplatelet therapy by a third at least.
PI13
ACUTE PROTECTIVE EFFECTS OF STATINS IN THE RAT MODEL OF RENAL ISCHEMIA-REPERFUSION INJURY: DIFFERENCE BETWEEN STATINS
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Renal dysfunction due to ischemia reperfusion (I/R) injury is problem following renovascular surgery or organ transplantation. Statins demonstrated so called pleiotropic effects, independent of lipid-lowering actions, such as: antiinflammatory, vasodilatory, antioxidative and cytoprotection. The aim of this study was to compare beneficial effects of acute treatments (5 min prior reperfusion) with same doses of liposoluble prodigal simvastatin and hydrophilic statin-pravastatin (1 mg/kg i.v.) on renal dysfunction following I/R injury in an experimental model.
Experimental model. Male Wistar rats were randomized into six groups (n = 6–13 per group): control (I/R+saline or 10% DMSO), Sham-operated+saline or 10% DMSO and I/R+ simvastatin or pravastatin (1 mg/kg, 5 min prior reperfusion). I/R injury was induced by clamping the both renal vascular pedicle for 45 min followed by 4 h of reperfusion (2 ml/kg/h). In all groups blood and urine samples were taken and analysis for markers of renal impairment. Also, both kidney of each animal were taken for histopathological evaluation.
Results: I/R injury caused significant renal dysfunction by increase of serum urea and creatinine levels and increase of fractional excretion of Na+. In pravastatin treated group all of these parameters of renal dysfunction were significantly lower (P < 0.05, for all mentioned parameters). Serum levels of aspartate aminotransferase and γ-glutamyltransferase, the most important parameters of reperfusion injury, were significantly lower in a group of animals treated with pravastatin compared with simvastatin treated group. Both statins, especially pravastatin significantly attenuated I/R-induced renal injury by improving histological score in comparison with simvastatin treated or untreated ones, but not abolished renal injury completely.
Conclusion: These results show that acute use of statins, especially pravastatin, may important play a role in modulating renal dysfunction and allowing earlier recovery from an I/R injury.

PI14
ETHANOL EXTRACT OF CURCUMA COMOSA ROXB. EXHIBIT PROPHYLACTIC EFFECT ON ATHEROSCLEROSIS IN CHOLESTEROL-FED RABBITS
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Introduction: Curcuma comosa Roxb., an indigenous medicinal plant containing phytoestrogens, has been reported to have hypocholesterolemic and anti-inflammatory effects. This study was aimed to examine the potential prophylactic effect of C. comosa ethanol extract on experimental atherosclerosis in rabbits.
Methods: Rabbits were fed 0.5% cholesterol, 0.5% cholesterol plus 5 mg/day of simvastatin, 0.5% cholesterol plus 100 mg/kgBW/day of C. comosa ethanol extract or normal rabbit chow for 12 weeks. Blood samples for cholesterol and estradiol determination were collected in monthly intervals. At the end of the study period, endothelium-dependent and -independent vascular function of isolated aortic rings in vitro and aortic plaques were assessed.

PI15
EFFECTS OF MORINGA OLEIFERA LAM. ON ATHEROSCLEROTIC DEVELOPMENT IN HYPERCHOLESTEROLEMIC RABBITS
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Introduction: Moringa oleifera Lam. is used as traditional medicine in Asia. Recent studies demonstrated its hypocholesterolaemic, diuretic and cardiotoxic effects. Atherosclerosis is a disease of blood vessels characterized by accumulation of the lipids and fibrous elements in the arteries. The aim of this study was to investigate the antiatherosclerotic activities of Moringa oleifera leaf extract.
Methods: Rabbits were fed with normal rabbit chow, normal rabbit chow plus 0.5% cholesterol, normal rabbit chow plus 0.5% cholesterol and simvastatin (5 mg/day), and normal rabbit chow plus 0.5% cholesterol and freeze-dried powder of Moringa oleifera leaf extract (0.1 g/kg/day). Plasma cholesterol levels were analyzed every 4 weeks. Twelve weeks after the treatment rabbits were sacrificed for the examination of vascular function and plaque formation.
Results: The results demonstrated that the Moringa oleifera leaf extract significantly reduced plaque formation and restored endothelium-dependent vascular relaxation without any effect to the cholesterol levels.
Conclusion: Our results suggested that the Moringa oleifera leaf extract had therapeutic potential for prevention of atherosclerosis.

PI16
METABOLIC EFFECTS OF THE PPARα AGONIST SAR351034 AFTER 2 WEEKS OF QD DOsing IN HEALTHY ELDERLY SUBJECTS
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Introduction: SAR531034 is a novel peroxisome proliferator-activated receptor α (PPARα) agonist which also has weak PPARγ agonist activity. It is active in the nanomolar range in dyslipidemia and diabetes animal models. The aim of the present study was to assess the safety, tolerability and metabolic effects of SAR531034 in healthy elderly subjects at different dose levels.
Materials and methods: In a placebo-controlled, parallel-group, escalating oral dose study, 47 subjects (mean age 71.5 years) received once daily either 5, 15, 75 or 150 mg SAR531034 (n = 9 per group) or placebo (n = 11 total) over 15 days. Subjects received a standardized moderate-fat diet throughout the in-house period, which comprised 5 days run-in, 15-days treatment, and 7 days follow-up.
The primary PD parameter was the change from baseline to Day 15 in the area under the 24-h serum triglyceride profile (TG-AUC) following a high-fat breakfast challenge at 2 h after dosing. Secondary parameters were the changes from baseline to Day 14 in fasting triglycerides, n-3-esterified fatty acids (NEFA), HDL and LDL cholesterol, Apolipoproteins -B,-A1 and -CII, high-sensitivity CRP, fasting blood glucose, and fasting insulin.

Results: SAR351034 was well tolerated when given as daily oral doses of 5, 15, 75 or 150 mg over 15 days to healthy elderly subjects. After 15 days on 75 and 150 mg SAR351034 once daily, TG-AUC after a high-fat breakfast showed mean (SD) decreases from baseline of 20.33% (20.57%) and 17.64% (13.95%), respectively, whereas it increased by 24.61% (21.74%) from baseline in the placebo group. After 5 and 15 mg daily, changes from baseline were only –1.06% (23.54%) and +1.68% (15.36%), respectively.

Fasting insulin on Day 14 decreased from baseline after 15, 75 and 15 mg SAR351034 daily: mean percent changes were –22.56% (27.95%), –42.44% (28.61%) and –24.35% (26.67%). Fasting insulin changed little from baseline in the 5 mg group (+5.89%, SD 55.04%) and the placebo group (+5.89%, SD 55.04%) and increased in the placebo group (+33.88%, SD 126.91%) and +53.88%, SD 126.91%.

After 15 days on 75 and 150 mg daily, changes from baseline were only –1.06% (23.54%) and +1.68% (15.36%), respectively.

Activation of PPARα agonist SAR351034 at dose levels of 75 and 150 mg improved the 24-h triglyceride profile following a high-fat breakfast challenge, as compared with placebo; smaller effects were also observed at 5 and 15 mg. Fasting insulin was decreased at dose levels 15, 75 and 150 mg over the 2-week treatment period. These results indicate that activation of PPARα has beneficial effects on lipid and glucose metabolism which could be useful in the treatment of dyslipidemia and Type 2 diabetes mellitus.

PI18
HIGH DIETARY FAT FEEDING DURING PREGNANCY ALTERS HEPATIC CYTOCHROME P450 EXPRESSION OF PROGENY IN MICE
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Hepatic Cytochrome P450 Expression of Progeny in Mice

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Introduction: Recently, various effects of high dietary fat intake during pregnancy on fetuses and newborns were reported. However, the effects on drug metabolizing enzymes were mostly unknown. In this study, the effects of high fat intake on the expression level and activity of hepatic Cytochrome P450 (CYP) were examined.

Materials and Methods: The parent mice were given either a normal feed (ND) or high-fat feed (HF) from the first day of gestation until delivery, and then the normal feed was given to both groups. The newborn mice were kept with ND parents for 3 weeks and weaned and given normal feed until 6-weeks old. The levels of mRNA expression of hepatic Cyp3a11, protein expression of Cyp3a, and metabolic activity of Cyp3a in microsomal fraction, along with the pharmacokinetics of triazolam were examined for 6-week-old mice. CAR expression level and activation of MAPK were also examined for 6-week-old mice.

Results: Decreases in the level of protein expression of hepatic Cyp3a and metabolic activity of Cyp3a in microsomal fraction were observed in the HF group. An increase in AUC and decrease in total clearance of triazolam were observed in the HF group. A decrease in the mRNA expression level of CAR and an increase in the phosphorylation of MAPK in the liver were observed in the HF group.

Conclusion: These results revealed that a high-fat intake during pregnancy results in a decrease in the expression level and activity of Cyp3a. These results also indicate the possibility that a decrease in the expression level of CAR by MAPK activation contributed to the decrease in the hepatic Cyp3a expression level.

PI19
EFFECT OF GEMFIBROZIL DOSE ON THE INACTIVATION OF CYP2C8 IN HUMANS
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Gemfibrozil is the most potent in vivo inhibitor of CYP2C8 known to date. The metabolite, gemfibrozil-1-O-glucuronide inactivates CYP2C8 rapidly (Honkalamm et al. Clin Pharmacol Ther 2011; 89 in press) and irreversibly; the low CYP2C8 activity lasts for several days after the usual 600 mg dose of gemfibrozil (Backman et al. Drug Metab Disp 2009; 37:2359-66). However, the effect of gemfibrozil dose on the extent of CYP2C8 inhibition is not known. We studied the effect of gemfibrozil dose on CYP2C8 activity using repaglinide as a probe drug. In a randomized, 5-phase cross-over study, ten healthy volunteers ingested 0.25 mg repaglinide 1 h after a single oral dose of 30, 100, 300 or 900 mg gemfibrozil or without gemfibrozil (control). Concentrations of plasma repaglinide, gemfibrozil and their metabolites, and blood glucose were measured up to 9 h. A gemfibrozil dose of 30, 100, 300 and 900 mg increased the mean (SD) AUC of repaglinide from the control of 4.7 (1.0) ng·h/ml to 8.3 (2.3), 21.2 (8.0), 31.5 (10.4) and 38.8 (11.4) ng·h/ml respectively (P < 0.001), and its Cmax from 4.3 (1.2) ng/ml to 5.6 (1.1), 6.9 (1.9), 8.8 (2.5) and 9.6 (2.2) ng/ml respectively (P < 0.05). The results are consistent with approximately 50% inhibition of CYP2C8 already with the 30 mg dose of gemfibrozil, and >95% inhibition with 900 mg gemfibrozil. In drug-interaction studies, a single 900 mg dose of gemfibrozil could be used instead of multiple gemfibrozil doses to achieve nearly complete inhibition of CYP2C8 activity.
**P120**

**EFFECT OF EARLY VS. DELAYED LEVODOPA/CARBIDOPA IN PARKINSON’S DISEASE**

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**Introduction:** Levodopa/carbidopa is the ‘gold standard’ treatment for Parkinson’s disease. However, after 4–5 years of treatment, efficacy is believed to decline and patients start to experience motor fluctuations and dyskinesias. The study aimed to explore the effect early vs. late levodopa/carbidopa initiation has in Parkinson’s disease. The aim was achieved by determining treatment initiation following the diagnoses and the side effects experienced after treatment initiation.

**Methods:** The methodology consisted of a semi-structured questionnaire survey and a review of patients’ medical files. The study population consisted of 15 patients in a Parkinson’s disease support group in Port Elizabeth, South Africa who were diagnosed with Parkinson’s disease by a neurologist and who were on levodopa/carbidopa therapy at the time of the study.

**Results:** More than 40% of patients (seven patients) experienced dyskinesia of which six patients had been on levodopa/carbidopa therapy longer than 10 years and one patient had their initiation delayed and had been on levodopa/carbidopa >5 years. The ‘wearing off’ phenomenon was experienced by only one patient who had been on levodopa/carbidopa for 7 years. In this patient, levodopa/carbidopa was initiated immediately after diagnosis was confirmed. ‘On-off’ phenomenon occurred in 53.3% of patients. All patients had been on levodopa/carbidopa longer than 5 years. Freezing episodes presented in 26.7% of patients.

**Conclusion:** No relationship between the initiation of levodopa/carbidopa therapy and the onset of side effects was found. However, it was found that after 5 years, patients did experience side effects associated with levodopa/carbidopa treatment.

**P121**

**5-AMINOLEVULINIC ACID AND HIGH ENERGY SHOCK WAVES FOR SONODYNAMIC THERAPY: EFFECTS ON SK-N-BE AND SH-SY5Y NEUROBLASTOMA CELL LINES**

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**Introduction:** An attractive form of treatment for solid tumors is sonodynamic therapy based on the ability of ultrasounds to generate acoustic cavitation and to activate a tumor-localizing sonosensitizer agent such as porphyrin compounds like 5-aminolevulinic acid (ALA). HESW, generated by a piezoelectric device, are able to induce acoustic cavitation, which results in a concentration of energy sufficient to generate a sonoluminescence emission, able to cause electronic excitation of porphyrins by energy transfer and initiate a photochemical process resulting in cytotoxic reactive oxygen species (ROS). To this purpose, we have investigated the ability of HESW to activate ALA in human neuroblastoma SK-N-BE and SH-SY5Y cell lines.

**Materials and Methods:** Cell cytotoxicity was measured with WST-1 proliferation assay and cell death was evaluated by flow cytometric analysis. The relationship between sonodynamic treatment and production of ROS was evaluated by flow cytometric analysis with dichlorofluorescein diacetate. Furthermore, mRNA expression of different genes involved in apoptosis through ROS production was evaluated by quantitative SYBR Green real time RT-PCR and fluorescence microscopic examination was carried out to highlight ROS production and cell death.

**Results:** We have identified different treatment schedule of ALA (50–300 μg/ml) and HESW (0.22–0.43 mJ/mm²; 500–1000 shots, 4 shots/s) to get the best cytotoxic rate in the two cell lines studied. Briefly, sonodynamic treatment was able to induce a significant decrease of cell growth compared to untreated cells at 72 h in both SK-N-BE and SH-SY5Y cells up to 35% and 50%, respectively. Exposure of ALA preincubated cells to HESW was able to increase significantly ROS production with different onset and extent in SK-N-BE and SH-SY5Y cells and the apoptotic rate was significant increased at 24 h in both cell lines (P < 0.01).

**Conclusion:** Our results show that HESW could be able to activate porphyrin compounds in neuroblastoma cell lines by acoustic cavitation obtaining a significant in vitro cytotoxicity through ROS production.

**P122**

**METFORMIN AMELIORATES COGNITIVE IMPAIRMENT AND OXIDATIVE STRESS IN THE HIPPOCAMPS OF RATS WITH CHRONIC CEREBRAL HYPOPERFUSION**

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There is an increased risk of developing vascular dementia (VD) among patients with type 2 diabetes. Insulin resistance and impairment in cerebral insulin receptor signaling may exacerbate ischemic neuronal damage by impairing cellular metabolism, increasing oxygen stress, and contribute to age-related cognitive deficits. Metformin, one of the most commonly drugs used for the treatment of type 2 diabetes, has been shown to activate AMPK and enhance insulin sensitivity. This study aimed to investigate the effect of metformin on spatial cognitive impairment and oxidative stress in the hippocampus induced by permanent occlusion of bilateral common carotid arteries in rats. Oral administration of metformin (250 mg/kg) significantly improved learning and memory deficits of rats induced by chronic cerebral hypoperfusion in Morris water maze task, increased the alternation percentage in alternation behavior task, and decreased wrong response times in passive avoidance test compared with model group. In addition, metformin also decreased the level of lipid peroxidation in the hippocampus of VD rats, improved mitochondrial enzyme activities of respiratory chain and energy metabolism, and attenuated the neuronal pathological alterations in the hippocampal CA1 area. The results presented the first evidence of a cognitive improving effect of metformin in the model of vascular dementia and diabetic cerebrovascular complications.

**P123**

**HIV ASSOCIATED DEMENTIA: ROLE OF NEUROSTEROIDS**

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**Background:** HIV-associated dementia (HAD), is a neurological complication of HIV infection itself and causes cognitive, motor and behavioral impairment. The HIV-proteins Tat and gp-120 can cause neurotoxicity directly and indirectly and implicated in HAD pathogenesis. The cognitive decline in HAD is correlated well with synaptic loss rather than neuronal loss. The exact mechanism of widespread synaptic loss in HAD remains elusive.

**Methodology:** A systematic pubmed research of published articles on HAD from molecular to epidemiological studies to identify the gaps in the pathogenesis.
Results: HIV-proteins are implicated to cause excitotoxicity via NMDA-receptors. The NMDA-receptor signaling involves increase in intracellular Ca^{2+} in neurons which activate nNOS to synthesize Nitric Oxide(NO). The NO activates Mitogen-associated protein kinase (MAPK). MAPK phosphorylates Microtubule-associated protein-2 (MAP2). MAP2 phosphorylation cause conformational changes, microtubular disassembly and MAP2 degradation via ubiquitin-proteosome pathway at various levels.

Discussion: Under physiological conditions NMDA mediated Ca^{2+} signaling also increase cholesterol transport into mitochondria, for steroidogenesis (Pregnenolone) by cytochromeP450 cholesterol side-chain cleaving enzyme (CYP11A1). Neurosteroid Pregnenolone binds to MAP2, inhibits its phosphorylation, decrease degradation and increase microtubule assembly. But NO has been shown to inhibit CYP11A1 in concentration dependent manner and reduce steroidogenesis. Since there is up-regulation of NO production from HIV infected microglia, circulating levels of NO is high. This can cause profound inhibition of steroidogenesis in the brain, hence predispose to increased MAP2 degradation and synaptic loss during Ca^{2+} signaling, even in absence of HIV proteins. Hence dietary pregnenolone supplements could have potential role in ameliorating cognitive decline in HAD.

Topic: NEURODEGENERATION (Poster)

P124
EFFECTS OF CALCIUM-CHANNEL BLOCKER NIFEDIPINE ON PENICILLIN-EPILEPSY-INDUCED HIPPOCAMPAL NEURONAL LOSS IN RATS
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Introduction: Epileptic seizures cause pathological changes such as sclerosis and pyramidal neuronal loss in the hippocampus. Experimentally, epilepsy can be induced by application of various chemicals (penicillins etc.) directly to the cerebral cortex. Nifedipine is a dihydropyridine type voltage-sensitive L-type calcium-channel blocker, inhibiting the transmembrane influx of calcium ions which are major signaling molecules. It has clearly been shown to have variable and even contradictory (beneficial or detrimental) effects in different studies and its neuroprotective or neurotoxic mechanisms remain to be clarified in other, still not investigated models, such as penicillin-epilepsy. Accordingly, the present study aimed at investigating the effect of nifedipine on hippocampal neuronal loss in penicillin-epilepsy model.

Methods: Epilepsy was induced in rats by intracortical application of 500 IU penicillin G, and the effect of nifedipine (10 mg/kg i.p.) on hippocampal pyramidal neuronal loss in CA1, CA2 and CA3 subfields (optical fractionator method- a stereological method) were investigated.

Results: Seizures related with penicillin-epilepsy in nifedipine groups rats were partly more manifest compared with control group. The number of neurons (>103) in the control group was (mean ± SD) 183.687 ± 3184. In the penicillin-epileptic rats, the neuron number was decreased to 146.318 ± 3042 (P < 0.05, Mann–Whitney U). Nifedipine, significantly decreased the neuron number to 128.873 ± 1157 (P < 0.05, Mann–Whitney U).

Conclusions: Calcium-channel blocker nifedipine increased the loss of hippocampal neurons and partly relieved epileptic seizures in penicillin-epileptic rats. Nifedipine could not protect against a variety of neurological insults including epilepsy.

P125
INTERACTION BETWEEN AN ANTIOXIDANT, TOCOPHEROL AND GABAPENTIN IN THE FORMALIN TEST
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Introduction: Tocopherol is one of the most common antioxidants and, perhaps, the one of the general public. Gabapentin is well-known potent analgesic in the treatment of neuropathic pain. Intrathecal tocopherol and gabapentin was shown previously the antinociceptive effect in formalin test. The present study was performed to investigate the interaction between intrathecal tocopherol and gabapentin in formalin rats.

Methods: Sprague-Dawley rats with lumbar intrathecal catheter were tested for their paw flinches by 5% formalin injection after intrathecal administration of tocopherol and gabapentin respectively. After obtaining dose-response curves for each agents, the effect of the combination (1/2, 1/4, 1/8 of 50% effective dose (ED50) in each agents) were tested by an isobolographic analysis using ED50. The total fraction value was calculated as (ED50 dose of tocopherol in combination)/(ED50 dose of tocopherol alone)+(ED50 dose of gabapentin in combination)/(ED50 dose of gabapentin alone). The experimental procedures were performed in accordance with the animal care guideline of the Korean Academy of Medical science.

Results: The combination produced significant decreases in the number of flinches in both phase 1 and 2 of the formalin test. ED50s of intrathecal tocopherol and gabapentin in the phase 2 were 17.6 mg/kg, 75.3 mg respectively. The ED50 values of the combination were significantly lower than the calculated additive values (P < 0.05) in phase 2. Total fractional dose value was 0.66 in the phase 2.

Conclusion: An antioxidant, tocopherol and gabapentin had synergistic antinociceptive effects on the inflammatory facilitated nociception in formalin rats.

P126
SYSTEMATIC QUALITY APPRAISAL AND RECOMMENDATION EXTRACTION FROM DEMENTIA GUIDELINES ON MANAGEMENT OF BEHAVIOURAL AND PSYCHOLOGICAL SYMPTOMS
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Introduction: Within the treatment of dementia, management of behavioural and psychological symptoms (BPSD) is an important but complex component. Our study goal was to appraise the quality of existing dementia guidelines; to extract practice recommendations for BPSD from selected guidelines; to select key practice recommendations for which there was agreement among guidelines.

Methods: We conducted a systematic search in MEDLINE and guideline organization databases supplemented by hand search of Web sites. Quality assessment was performed by 2 independent reviewers using the Appraisal of Guidelines Research & Evaluation instrument (AGREE). Specific practice recommendations (SPRs) for BPSD were extracted and compared for their level of evidence and strength. We developed a systematic approach to determine for which recommendations agreement was found.

Results: Fifteen guidelines were eligible for quality appraisal by AGREE of which 5 were selected for recommendation extraction. We extracted 18 SPRs on management of BPSD, but variation in grading systems (none using GRADE) complicated interpretation, comparison and generalisability of recommendations among guidelines. By introducing a posteriori defined criteria, we determined a level of agreement for each SPR, and selected key practice recommendations for which there was agreement. For most non-pharmacological interventions no agree-
ment among SPRs for BPSD was found. Pharmacological SPRs were recommended as second-line treatment, with guidance for timely discontinuation.

Conclusion: More primary evidence and guidelines for management of BPSD are needed. Valid methods to deal with absence of agreement among guidelines in the appraisal of the level of evidence and strength of practice recommendations should be further explored.
PI127
MONITORING OF IMATINIB CONCENTRATION IN CML PATIENTS BY HPLC METHOD
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Introduction: Imatinib is a 2-phenylaminopyrimidine derivative that functions as a specific inhibitor of the oncopgenic tyrosine kinase Bcr/Abl. It is used in chronic myelogenous leukemia (CML), gastrointestinal stromal tumors (GISTs) and a number of other malignancies. Imatinib is primarily metabolized to its active metabolite, an NB-demethylated piperazine derivative. Metabolism of imatinib occurs in the liver by several isozymes of the cytochrome P450. The major route of elimination is in the bile and feces. The half-lives of imatinib and its main metabolite are 18 and 40 h, respectively.

Materials and Methods: The aim of this study was to determine plasma imatinib concentration in CML patients. Daily dose was 400 mg imatinib. Blood sample was collected 24 ± 3 h after drug administration. The method used was high performance liquid chromatography (HPLC). Imatinib was extracted from plasma with methanol. Clozapine was used as an internal standard. The sample was fractionated on a column MN EC Nucleosil 1005-C18 EC 250 × 4.6 mm with a mobile system consisting of ammonium acetate buffer, methanol and acetonitrile (40:40:20). The flow rate was 0.75 ml/min. Quantitation was performed by measurement of UV detector at the wavelength of 265 nm.

Results: Imatinib levels in CML patients ranged from 230 to 4247 ng/ml. Mean imatinib concentration was 1010 ng/ml (943–1246 ng/ml) in 10 patients, and 1985 ng/ml (1879–2564 ng/ml) in six patients. Three patients had markedly low imatinib level: 4247 ng/ml.

Conclusion: HPLC-UV is a simple and reliable method for imatinib determination. Results demonstrated large intra- and interindividual differences, and indicated the need for monitoring imatinib concentration.

PI128
HEMENE OXYGENASE-1 PROTECTS ACUTE MYELOID LEUKAEMIA CELLS FROM FRONT LINE CHEMOTHERAPEUTIC DRUGS
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Introduction: Heme oxygenase-1 is fast becoming a pro-tumoral target in the treatment of human cancer. Currently little is known about HO-1 and its role in regulating apoptosis in human acute myeloid leukaemia (AML) in response to chemotherapy. Recently we have shown HO-1 protects AML samples from TNF induced apoptosis, it is regulated by a number of transcription factors, NRF2, NF-κB and activator protein-1 (AP-1). This study aims to analyse the role of HO-1 in regulating apoptosis in AML cells in response to two front line chemotherapy agents, daunorubicin and cytarabine.

Methods: qRT-PCR, western blot analysis and flow cytometry were used to detect the presence of either HO-1, Reactive oxygen species or cell death.

Results: This study show that HO-1 expression in AML samples was increased in response to both cytarabine and daunorubicin treatment. Cell death assays showed that most AML cells were relatively resistant to cytarabine, but less so, too daunorubicin. Further work showed that silencing HO-1 expression in combination with either daunorubicin or cytarabine induced a greater apoptotic response in these AML cells. Moreover, we showed that both daunorubicin and cytarabine induced apoptosis in AML by a ROS dependent mechanism. ROS also induced HO-1 expression, which subsequently provided resistance to cytarabine and daunorubicin treatment.

Conclusion: These findings suggest inhibiting HO-1 expression in conjunction with chemotherapeutic treatment could increase the number of cases who reach complete remission.

PI130
REGULATION OF HEME OXYGENASE-1 (HO-1) BY GENE PROMOTER METHYLATION IN ACUTE MYELOID LEUKAEMIA
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Introduction: Acute myeloid leukaemia (AML) is a heterogeneous disorder of haematopoietic stem cells (HSC), characterized by proliferation and differentiation of abnormal cells, to cause immature myeloid cells from bone marrow to grow. Recently, the role of cytoprotective genes in different types of cancers has been of interest, including HO-1, the rate-limiting enzyme of heme catabolism, as well as other Nrf2-dependent antioxidant enzymes. Cancer cells are often associated with focal hypermethylation of specific gene promoters organised as CpG islands. Here we tested the regulation of HO-1 expression by investigating the role of DNA methylation and chromatin related factors in human AML cancer cells.

Materials and Methods: Human AML cell line (THP-1) cells were used as a model of human AML. Cells were treated with the DNA methyl transferase (DNMT) inhibitor, 5-aza-2′-deoxycytidine (DAC) and the histone deacetylase (HDAC) inhibitor, trichostatin A (TSA), alone or in combination. The HO-1 levels were assessed by real-time PCR.

Results: Treatment of cells with DAC caused a 3.5-fold induction of HO-1 mRNA in a concentration-dependent manner. Use of the HDAC inhibitor alone had little effect on HO-1 levels, whereas its use in combination with DAC caused a synergistic induction in HO-1 levels, by over five-fold. Similar induction was observed with NAD(P)H dehydrogenase quinine oxidoreductase 1 (NQO1) mRNA levels. Other control genes were not regulated in this fashion.

Conclusions: Overall, these results suggest that there is a general deregulation of CpG island methylation in the promoter regions of HO-1 and NQO1. On-going research is investigating the impact of this epigenetic alterations in AML cells.

Acknowledgement: This work was supported by a project grant from the Big C Trust.

PI132
MONITORING OF SIDE EFFECTS OF CITOTOXIC DRUGS IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKAEMIA TREATED BY ALL IC-BMF 2002 PROTOCOL
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Introduction: The consolidation therapy of acute lymphoblastic leukaemia (ALL), in children with standard or intermediate risk, according to the protocol ALL IC BMF-2002, includes the application of cytostatics –
4 medium-high doses of methotrexate, (2 g/m², during 24 h), and 6-mercaptopurine during 56 days. For the drug detoxification was used calcium folinate. Today, 80% of children with this disease is cured, but resistance to the therapy and its toxic effects remain as serious clinical problem.

**Methods:** The aim of the study was monitoring of side effects in children with ALL, who received the treatment at the Department of Hematology, Institute for Health Protection of Children and Youth of Vojvodina in Novi Sad. Side effects were followed by a modified form of monitoring of acute toxicity of therapy. The study included children with acute lymphoblastic leukemia that during the period from 01.07.2010. to 31.12.2010.

**Results:** During this period, five patients which received consolidation therapy were followed. Thrombocytopenia occurred as the most frequent side effect. In two patients, the treatment with methotrexate did not cause significant change in platelet count. In another three patients, decreased platelet count after the administration of methotrexate was detected. Thrombocytopenia returned to the normal values after the application of calcium folinate. In addition, in one patient reported gastrointestinal toxicity, febrile condition and respiratory infection.

**Conclusion:** Side effects occurred after use of medium-high doses of methotrexate were mild to moderate and had a reversible character. The application of calcium folinate as an antidote, prevented severe side effects and intolerance of methotrexate.
HEMOSTASEOLOGY

P129
PHARMACODYNAMIC PARAMETERS OF SULFATED CELLULOSE FROM GOSSIPUM COTTON AT INTRAVENOUS ADMINISTRATION TO RABBITS
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Introduction: Researching of new anticoagulants (AK) of direct action among polysaccharides’s derivatives of bacterial, plant or animal origin is carried out strongly in many countries. These AK along with antiplatelet drugs PGE2 is known to activate four receptors EP1, EP2, EP3 and EP4. The effect of PGE2 is dependent on its concentration wherein lower concentrations of PGE2 via activation of the EP receptor enhance platelet aggregation, while higher concentrations inhibit aggregation. We investigated the role of the PGE2 receptor-EP4 in the inhibition of platelet aggregation, as this could be a potential target for antiplatelet therapies.

Methods: Platelet aggregation assays were performed ex vivo using a platelet aggregation analyser. Blood from healthy human donors was used to obtain platelet-rich plasma. Aggregation was induced using ADP. Different agonists and antagonists were added to investigate their effects on platelet aggregation. Ca2 + flux changes caused by addition of agonists were also examined using a fluorescent Ca2 + dye (Fluo-3) by flow cytometry. Flow cytometry was used to ascertain expression of EP4 receptor, P-selectin and GPIb/IIIa heterodimerization.

Results: We observed that in human platelets the EP4 agonist potently inhibited the platelet aggregation induced by ADP and this could be completely reversed by using an EP4 antagonist. Interestingly, the inhibitory effect of the EP4 agonist was brought about by protein kinase C but not adenylyl cyclase, accompanied by attenuated Ca2 + flux, decreased activation of glycoprotein IIb/IIIa and down-regulation of P-selectin. Most importantly, in vitro thrombosis formation was effectively reduced by the EP4 agonist and this effect was reversed using the EP4 antagonist.

Conclusions: These findings indicate that the EP4 receptor is a potential biological drug target in antiplatelet therapy.

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P134
IMPACT OF CYP2C9 POLYMORPHISMS AND/OR INHIBITORS ON THE RISK OF OVERANTICOAGULATION IN PATIENTS STARTING AN ACENOCOUMAROL TREATMENT
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Introduction: Aacenocoumarol is an oral anticoagulant commonly prescribed in Switzerland. As warfarin, the response to treatment varies widely and is affected by genetic and environmental factors, bleeding being the most frequent serious adverse event. Drug-drug interactions (DDIs) or CYP2C9 polymorphisms are among the factors known to affect the risk of overanticoagulation. The objective of this study was to investigate the impact of CYP2C9 polymorphisms and drug interactions on overanticoagulation risk in patients treated with aacenocoumarol.

Method: A prospective observational study was performed on patients starting aacenocoumarol. CYP2C9 genotypes were assessed and data on INR, comedinations, comorbidities, and doses of aacenocoumarol were collected during the first 35 days of therapy. Drugs known to inhibit CYP2C9 were considered as relevant DDIs. Overanticoagulation was defined as the occurrence of at least one INR>4.

Results: The CYP2C9 genotype of 107 subjects was assessed: 63.5% were wild-type subjects, 26.2% were carriers of CYP2C9*2 and 10.3% were carriers of CYP2C9*3. 42.1% had at least one INR>4. comedications, comorbidities, and doses of aacenocoumarol were collected during the first 35 days of therapy. Drugs known to inhibit CYP2C9 were considered as relevant DDIs. Overanticoagulation was defined as the occurrence of at least one INR>4.

Conclusion: In the first month of aacenocoumarol treatment, the presence of at least one allelic variant of CYP2C9 and/or prescription of CYP2C9 inhibitors expose patients to an increased risk of overanticoagulation. The findings of these studies support that CYP2C9 genotyping could be useful to identify patients requiring a closer monitoring.

P131
PG E2 INHIBITS THROMBOGENESIS VIA THE EP4 RECEPTOR IN A PROTEIN KINASE C MEDIATED PATHWAY
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Introduction: Acute myocardial infarction is one of the leading causes of death in the world which is caused by coronary artery thrombosis. Platelets play a central role in cardiovascular thrombosis. An important component of vascular diseases is inflammation. During inflammation prostaglandins (PG) like PGI2, PGE2 and PGD2 are released. In addition to the well known key pathways in platelet activation that are targeted by anti-platelet drugs PGE2 also affects platelet aggregation. PGE2 is known to activate four receptors EP1, EP2,
This work was supported by the Medical Directorate, University Hospitals of Geneva, Switzerland.

P135
ACENOCOUMAROL INTERACTIONS IN EVERYDAY PRACTICE
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Introduction: Drug-drug interactions are great of importance, but poorly known in everyday practice. Oral anticoagulant agents have a very narrow zone between therapeutic and potentially dangerous ranges, interfere with diet and have multiple drug interactions. In Hungary acenocoumarol is much more preferred to warfarin when it comes to oral anticoagulation. There are no previous Hungarian data about acenocoumarol’s potential to interact with other agents.

Methods: We reviewed 2010’s patients with International Normalised Ratio (INR) four or above at the 1st Department of Medicine, Semmelweis University, Budapest. Drug additions, withdrawals, switches and dosage modifications were monitored in order to investigate possible coumarine interactions. We used Drug Interaction Probability Scale (DIPS) (1) to confirm our speculations.

Results: Using the criteria above we found that out of 4261 inpatients in year 2010 at this hospital, 137 cases (3.22%) with increased INR results were reported. After the evaluation, 45 interactions were suspected for the cause of INR elevation, which is more than one third (34.3%) of these patients. We found that the most common drugs to interact with acenocoumarol were statins (12 cases), quinolones (11 cases) and proton-pump inhibitors (PPIs) (four cases). We are also presenting the case of an 83-year-old acenocoumarol-receiving patient, who switched 10 mg atorvastatin therapy to 20 mg simvastatin by mistake which was resulted in INR over 6 and a large hematoma on her left shin.

Conclusions: Coumarine interactions represent more than 1% of 2010’s clinical cases at this university hospital. In every case of unidentified INR elevation is suggested to consider drug-drug interactions in the background. When adding statins, quinolones or PPIs to patients receiving acenocoumarol, more frequent monitoring and if necessary coumarine dosage modifying is recommended to prevent hemorrhages due to high INR.

Reference:

P136
THROMBOLYSIS FOR ACUTE ISCHAEMIC STROKE: A PROSPECTIVE ANALYSIS OF RESOURCE CHALLENGES ASSOCIATED WITH PROVIDING A REGIONAL SERVICE
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Introduction: Thrombolytic therapy is the only pharmacological treatment licensed for acute ischaemic stroke. The Western Infirmary Glasgow provides a regional stroke thrombolysis service covering five acute hospitals. We examined the clinical burden created by the service.

Methods: We conducted a prospective analysis of referrals from other hospitals over 6 weeks.

Results: Thirty-three patients were referred between 14/10/10 and 25/11/10: 20 (61%) were accepted and 8 (24%) received thrombolysis. 13 referrals (39%) were not accepted: 7 (21%) had comorbidity-related contraindications to thrombolysis and 6 (18%) were outwith the 270-min time-window. Twelve patients (36%) were transferred to our hospital but not treated: 5 (15%) had comorbidity-related contraindications to thrombolysis which were not apparent before transfer. Four (12%) had radiological contraindication and 4 (12%) had symptom resolution.

Baseline brain imaging was performed at the Western Infirmary for 17 of the 20 patients transferred (85%) and at the original hospital in 2 (10%). Brain imaging was not indicated in one patient who was transferred. Further investigation (brain imaging, carotid Doppler, TCD microbubble test, 24-h electrocardiography or echocardiography) was performed in 12 of the 20 patients transferred (60%) before repatriation to their local hospital. The median delay before repatriation to a patient’s local hospital was 2 (0–4) days.

Conclusions: Less than one-quarter of patients referred for consideration of thrombolysis receive it. More than one-third of patients referred were transferred inappropriately. There can be delay to repatriation and significant resource is required to support this service. Improved identification of suitable patients for transfer is needed.

P137
SYSTEMATIC REVIEW OF PHARMACOKINETIC OF FACTOR IX
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The substitutive therapy of hemophilia B relies approximately for half on recombinant and plasma derived products, on a worldwide basis. More than 10 years after the first marketing authorization of recombinant factor IX, the quantities used have more than doubled without significant change in therapeutic practices. It seems that the number of patients treated cannot explain such a difference. It raises the question of real pharmacokinetic equivalence or not between plasma-derived products and the recombinant one. Indeed, in 2003 Kisker et al. already suggested that, based on pharmacokinetics modelling, therapy using recombinant factor IX may be more expensive. This prompted us to review all available data. Systematic review of PubMed indexed literature has been performed, leading to the identification of 51 studies. Seven studies on animals and two on continuous infusion were not integrated, yielding to 42 analysed studies. Among these studies only 15 (36%) were in accordance with international sampling recommendations and 19 (45%) were conform to recommendations of doses for injection. Concerning recovery (IU/dl/IU/kg), only 20/42 were considered as methodologically acceptable; with data available for 26 studies for plasma-derived FIX (1.17 ± 28) vs rFIX (0.81 ± 0.14) showing a significant advantage for plasma-derived FIX (P < 0.0001). Only four paired studies were available, showing also a very clear difference between 1.41 ± 0.33 for plasma-derived FIX and 0.82 ± 0.05 for rFIX P = 0.03. The comparison of clearance (ml/h/kg) could be achieved among 22 studies for pdFIX and only three studies for rFIX, showing also a significant difference: 5.6 ± 1.9 for pdFIX and 8.5 ± 4.5 for rFIX with P < 0.05. These data may explain why lower quantities may be enough for treating hemophilia B patients when using pd-FIX as previously suggested.

P138
RESVERATROL ENHANCES ANTIAGGREGATORY ACTIVITY OF ACETYLSALICILIC ACID AND REDUCES ITS GASTROTOXICITY IN PATIENTS WITH CORONARY HEART DISEASE
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Introduction: Coronary heart disease (CHD) has been and remains a major contributor to morbidity and mortality in developed countries. Its etiology is closely related to platelet dysfunction. Abnormalities of platelet adhesion and aggregation may contribute to the development of atherosclerosis, proliferation of smooth muscle cells, and acute thrombosis. Effective anti-platelet treatment can reduce the risk of thromboembolism. It has long been known that moderate drinking of red wine reduces mortality and morbidity of CHD (‘French paradox’). Further studies showed...
that resveratrol, a polyphenolic compound existing in red wines, was the major active component in decreasing the incidence of CHD. Aim of this study was to evaluate effects of resveratrol on platelet function and gastric mucosa in CHD patients receiving basic antiplatelet therapy with acetylsalicylic acid (ASA).

**Methods:** After exclusion criteria, CHD patients were randomized to treatment with either ASA 75 mg/day (n = 10) or ASA 75 mg/day + resveratrol 50 mg/day (n = 10) in addition to their basic antiinflammatory therapy for 28 days. Platelet optical aggregometry test was carried out by G. V. Born turbidimetric method and using the solutions of collagen 2 mg/ml and adenosine diphosphate (ADP) 20 μmole/l as inducers. Systemic production of thromboxane A2 was estimated by its main urinary metabolite (11-dehydro-thromboxane B2) level (11-dehydro-TxB2 ELISA kit, Assay Designs, USA). Ulercogenic action of investigated drugs was estimated by gastroduodenoscopy and expressed as gastric ulcerative index. All tests were performed twice: before and after of 28-days treatment course.

**Results:** Co-administration of resveratrol led to intensification of antiaggregative effects of ASA. So, degree and rate of collagen-induced aggregation in ASA+resveratrol group (II) in comparison with ASA group (I) were decreased by 31.3% and 20.8% (P < 0.05), ADP-induced – by 37.5% and 29.1% (P < 0.05) accordingly. Urine 11-dehydro-TxB2 level in group II was 43.7% (P < 0.05) down from group I. Co-administration of resveratrol also led to 1.47-fold (P < 0.05) decreasing of gastric ulcerative index.

**Conclusions:** Thus, resveratrol possesses a considerable antiaggregatory and gastroprotective action. Its co-administration in ASA-receiving CHD patients intensifies antiaggregatory and decreases ulercogenic properties of ASA.

**PI139**

**THIOTRIAZOLIN ENHANCES ANTIAGGREGATORY ACTIVITY OF ACETYLSALICILIC ACID AND REDUCES ITS GASTROTÓXICITY IN PATIENTS WITH CORONARY HEART DISEASE.**

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**Introduction:** Coronary heart disease (CHD) has been and remains a major contributor to morbidity and mortality in developed countries. Its etiology is closely related to platelet dysfunction. Abnormalities of platelet adhesion and aggregation may contribute to the development of atherosclerosis, proliferation of smooth muscle cells, and acute thrombosis. Effective anti-platelet treatment can reduce the risk of thromboembolism. There is mounting evidence that antioxidants may help to prevent CHD and diminish thrombotic events. However, effects of antioxidants on platelet function in vivo are controversial. Aim of this study was to evaluate effects of novel antioxidant thiottiazolin (morpholinium 3-methyl-1,2,4-triazoline-5-thioacetate) on platelet function and gastric mucosa in CHD patients receiving basic antiplatelet therapy with acetylsalicylic acid (ASA).

**Methods:** After exclusion criteria, CHD patients were randomized to treatment with either ASA 75 mg/day (n = 10) or ASA 75 mg/day + thiottiazolin 100 mg/day (n = 10) in addition to their basic antiangiinal therapy for 28 days. Platelet optical aggregometry test was carried out by G. V. Born turbidimetric method and using the solutions of collagen 2 mg/ml and adenosine diphosphate (ADP) 20 μmole/l as inducers. Systemic production of thromboxane A2 was estimated by its main urinary metabolite (11-dehydro-thromboxane B2) level (11-dehydro-TxB2 ELISA kit, Assay Designs, USA). Ulercogenic action of investigated drugs was estimated by gastroduodenoscopy and expressed as gastric ulcerative index. All tests were performed twice: before and after of 28-days treatment course.

**Results:** Co-administration of thiottiazolin led to intensification of antiaggregative effects of ASA. So, degree and rate of collagen-induced aggregation in ASA+thiottiazolin group (II) in comparison with ASA group (I) were decreased by 22.5% and 41.3% (P < 0.05), ADP-induced – by 38.9% and 19.7% (P < 0.05) accordingly. Urine 11-dehydro-TxB2 level in group II was 35.3% (P < 0.05) down from group I. Co-administration of thiottiazolin also led to 1.72-fold (P < 0.05) decreasing of gastric ulcerative index.

**Conclusions:** Thus, thiottiazolin possesses a considerable antiaggregatory and gastroprotective action. Its co-administration in ASA-receiving CHD patients intensifies antiaggregatory and decreases ulercogenic properties of ASA.

**PI140**

**THROMBOMODULIN AND PAI-1 EXPRESSION IN RECURRENT MISCARRIAGE.**


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**Introduction:** Many studies have indicated the role of hereditary and acquired thrombophilia in recurrent miscarriage. Thrombomodulin and PAI-1, two molecules interfering with coagulative and fibrinolytic pathways, are expressed mainly on the endothelium and the placental cytotrophoblast.

**Methods:** Histological specimens from 10 miscarriages and five elective abortions, were obtained during the first trimester of gestation to investigate differences in expression of thrombomodulin and PAI-1 in trophoblastic cells at the implantation site and at the decidua basalis. Specimens were appropriately prepared for microscopic analysis, following immunohistochemical technique.

**Results:** In the miscarriage group, thrombomodulin expression was low, regarding density and low up to moderate, regarding intensity at the implantation site, whereas negative up to moderate, concerning density and low up to moderate, concerning intensity at the decidua basalis. In the elective abortion- control group, at the implantation site, density was strong and intensity low up to strong. No expression was detected at the decidua basalis. In the miscarriage group, PAI-1 expression exhibited low density and low up to moderate intensity at the implantation site, whilst negative up to moderate density and low up to moderate intensity at the decidua basalis. In the elective abortion group, density was low up to moderate and intensity low up to strong at the implantation site. Finally, at the decidua basalis, density was low up to moderate and intensity was moderate.

**Conclusions:** Low thrombomodulin and PAI-1 expression in trophoblast might constitute a suspending factor to normal development and coagulation status of the placenta, thus suggesting a contribution to the phenomenon of recurrent miscarriage.
CARDIOVASCULAR DISEASES: HEART

P141
UTILISATION OF THROMBOPROPHYLACTIC AGENTS IN NORTH ESTONIA MEDICAL CENTRE SURGERY CLINIC 2008–2010: IMPACT OF CHANGES IN LOCAL GUIDELINE RECOMMENDATIONS
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Introduction: Patients undergoing surgery are exposed to risk developing deep venous thrombosis (DVT). Dislodged thrombi may travel to lungs and cause pulmonary embolus (PE). The extent of risk of DVT depends on the type of surgery performed. In Estonia mainly low molecular weight heparins (LMWH) are used for DVT prophylaxis in surgical patients. For orthopaedic patients new oral anticoagulants rivaroxaban and dabigatran are available in Estonia. Dabigatran and LMWHs bemiparin, dalteparin, enoxaparin and nadroparin are reimbursed for hip and knee replacement surgery patients for ambulatory use after hospital discharge. In North Estonia Medical Centre (NEMC) enoxaparin and dabigatran are recommended for DVT prophylaxis in surgical patients.

In 2007 survey was conducted in NEMC surgical wards to evaluate appropriateness of DVT prophylaxis with nadroparin. We included patients from surgical wards whom surgery was performed during November 2006. The survey showed underuse of prophylaxis and wide use of incorrect dosages of nadroparin. After that local recommendations have been updated and since 2008 enoxaparin and from 2010 dabigatran are used for DVT prophylaxis.

Purpose of this study was to evaluate consumption of DVT prophylactic drugs enoxaparin and dabigatran in NEMC surgery clinic during 2008–2010.

Methods: Drug utilisation study was performed in NEMC surgery clinic during years 2008, 2009 and 2010. Prescribed daily doses (PDD) of enoxaparin and dabigatran were obtained from hospital pharmacy database. Information about hospital days and number of surgeries performed in surgery clinic during 2008, 2009 and 2010 were obtained from hospital databases.

Results: There were 18400, 35602 and 36900 PDDs of studied drugs used in years 2008, 2009 and 2010, respectively. As background information we checked hospital days and number of surgeries performed during 2006 till 2010. From that information conclusion was made that surgical activity and patient population according to the DVT risk category had remained similar as in 2007.

Conclusion: Decision to use enoxaparin instead of nadroparin has increased adherence to the local guideline recommendations for DVT prophylaxis in surgical patients in NEMC. Addition of dabigatran to armamentarium of DVT prophylactic agents in 2010 has also positive impact for sufficient usage of pharmacologic measures for DVT prophylaxis among surgical patients.

However, an audit of medical records of surgical patients may be needed to give definite judgement that also other DVT prophylactic methods are properly used, also taking into account special patient subpopulations (e. g renal failure patients).

P142
CHANGES IN THE PRESCRIPTION OF RECOMMENDED DRUGS FOR ACUTE MYOCARDIAL INFARCTION IN ESTONIA IN 2001 VS. 2007
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Introduction: Current acute myocardial infarction (AMI) guidelines recommend the use of platelet aggregation inhibitors, beta-blockers, angiotensin converting enzyme inhibitors (ACEI) or angiotensin II receptor blockers (ARB) and statins for in-hospital and long-term treatment. The aim of the study was to evaluate the changes in the prescription of these drugs in Estonian hospitals in 2001 vs 2007.

Patients and Methods: We performed a retrospective cross-sectional study including random samples of hospitalized AMI cases. Chi-square test and logistic regression was used to study the changes in the prescription of the drugs.

Results: Final analysis included 423 cases in 2001 and 687 cases in 2007. The prescription rates of studied drugs for in-hospital and for outpatient use were significantly higher in 2007 (Table 1). Compared to 2001, in 2007 more patients were prescribed drugs from all five drug (sub) groups during hospitalization (3.3% vs 18.5%, P < 0.001) and for outpatient use (3.8% vs 22.2% P < 0.001). In multivariate analysis, >75-year-old patients, those with ST-segment elevation AMI, and those on warfarin therapy were less likely to be prescribed drugs for AMI.

Table 1. Prescription rates of recommended drugs in Estonia.

<table>
<thead>
<tr>
<th>Drug class</th>
<th>Year 2001</th>
<th>Year 2007</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>During hospitalization</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspirin</td>
<td>87.7</td>
<td>90.1</td>
<td>0.212</td>
</tr>
<tr>
<td>Clopidogrel/Ticlopidine</td>
<td>8.5</td>
<td>34.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Beta-blockers</td>
<td>77.8</td>
<td>80.1</td>
<td>0.363</td>
</tr>
<tr>
<td>ACEI/ARB</td>
<td>53.7</td>
<td>68.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Statins</td>
<td>16.1</td>
<td>48.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Out-patient use</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspirin</td>
<td>82.6</td>
<td>88.5</td>
<td>0.013</td>
</tr>
<tr>
<td>Clopidogrel/Ticlopidine</td>
<td>9.9</td>
<td>41.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Beta-blockers</td>
<td>70.1</td>
<td>80.4</td>
<td>0.001</td>
</tr>
<tr>
<td>ACEI/ARB</td>
<td>52.6</td>
<td>73.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Statins</td>
<td>23.5</td>
<td>57.6</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Conclusions: The prescription of recommended drugs for AMI has increased in Estonia, still only a fifth of the patients are prescribed drugs from all five drug (sub) groups.
P143
INTERACTIONS BETWEEN CES2, CYP2C9, UGT1A6 AND COX1 GENETIC VARIANTS AND ACETYLSALICYLIC ACID MODIFY THE RISK OF MYOCARDIAL INFARCTION

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Introduction: To investigate whether genetic variants in the enzymes CES2, CYP2C9, UGT1A6, COX1 and COX2 modify the effectiveness of acetylsalicylic acid (ASA) therapy in the prevention of myocardial infarction (MI).

Methods: In a population-based registry of pharmacy records linked to hospital discharge records (PHARMO RLS), a nested case-control study was performed. Cases had a first MI between 1991 and 2005, controls were matched to MI cases by age, gender and region. Patients were genotyped for tagging SNPs in genes coding for CES2, CYP2C9, UGT1A6, COX1 and COX2. Logistic regression was used to assess the interaction between ASA and genetic variants on the risk of MI and to adjust for confounding.

Results: The influence of 22 tagging SNPs was assessed in 853 cases and 887 control subjects. ASA-use was associated with a reduced risk of MI (adjusted odds ratio (ORadj) 0.74 (95% CI 0.56–0.97), P = 0.032). The CES2 rs11568311 and CYP2C9 rs1057910 variants were found to interact with ASA treatment (adjusted synergy index (SIadj) 0.43 (0.21–0.82), P = 0.023). No significant interactions between other genetic variants and barium-chloride (BaCl2, 1 mM), a blocker of inward-rectifier K+ channels, antagonized the response to resveratrol (EC50 = 28.0 μM, respectively). Tetraethylammonium (TEA, 10 mM), which predominantly inhibits Ca2+-activated K+ channels and barium-chloride (BaCl2, 1 mM), a blocker of inward-rectifier K+ channels, antagonized the response to resveratrol (EC50 = 28.0 μM and 50.6 μM, respectively). The high concentration of resveratrol (>30 μM) relaxed HUV bathed by a medium containing 100 mM K+, with maximum response of 94% and EC50 of 47 μM, P < 0.05).

Conclusions: These results suggest that resveratrol induced endothelium-independent vasorelaxation of HUV. The glibenclamide-, 4-AP, TEA- and BaCl2-sensitive K+ channels are involved in vasodilatory effect. However, it seems that resveratrol has additional K+ channel independent mechanism of action.

Funding: Our work has been supported by Scientific Research Grant (TR31020) from the Ministry of Science (Serbia).

P144
CONFIRMATION OF QT PROLONGATION BY ORAL MOXIFLOXACIN IN HEALTHY JAPANESE SUBJECTS FOR THOROUGH QT/QTc STUDIES IN JAPAN
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Introduction: Thorough QT/QTc studies are being performed to assess cardiac safety of drugs in clinical developments. Moxifloxacin (Mox) is widely used as a positive control to demonstrate assay sensitivity of a study. However data in Japanese are lacking, while there is a possibility of ethnic difference in drug induced QT prolongation. Therefore we performed a study of QT prolongation by Mox in healthy Japanese volunteers.

Subjects and Methods: Forty healthy male subjects aged to y. o. were randomized either to 400 mg of Mox or placebo according to 2 way design. Plasma Mox concentrations were measured on the same schedule as administration day. The study was approved by the IRB of Kitasato University East Hospital.

Results: Thirty nine subjects finished the study and tolerated well. Plasma concentration of Mox was identical to reported data. QT/QTc prolongations were as large as 10.2 to 14.0 ms according to correction methods after Mox when compared to placebo. The prolongation correlated with plasma concentration of Mox.

Conclusion: Mox can be used as a positive control to assure the assay sensitivity in QT/QTc studies since it prolong QT intervals in Japanese healthy subjects.

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P145
THE EFFECT OF RESVERATROL ON THE HUMAN UMBILICAL VEIN WITHOUT ENDOTHELIUM
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Introduction: There are evidences that resveratrol induces vasorelaxation through both endothelium-dependent and endothelium-independent mechanisms. The effect of resveratrol on human umbilical vein (HUV) is not known. Therefore, the aim of our study was to define the role of different K+ channel subtypes in the vasodilatation of HUV induced by RSV.

Materials and Methods: Serotonin (5-HT) or 100 mM K+ were used for precontraction of the vein rings without endothelium. The cumulative concentration-response curves were obtained by adding increasing concentrations (1–100 μM) of resveratrol. A different K+ channel inhibitors were added in the bath before resveratrol in order to tested the role of vascular K+ channels in its effect.

Results: Resveratrol induced concentration-dependent vasodilatation (EC50 = 16.5 μM). A selective blocker of ATP-sensitive K+ channels, glibenclamide (10 μM) and 4-aminopiridine (4-AP, 1 mM), a non-selective blocker of voltage-gated K+channels, induced significant shift to the right (P < 0.05) of the concentration-response curves for resveratrol (EC50 = 38.0 μM and 49.0 μM, respectively). Tetraethylammonium (TEA, 10 μM), which predominantly inhibits Ca2+-activated K+ channels and barium-chloride (BaCl2, 1 mM), a blocker of inward-rectifier K+ channels, antagonized the response to resveratrol (EC50 = 28.0 μM and 50.6 μM, respectively). The high concentration of resveratrol (>30 μM) relaxed HUV bathed by a medium containing 100 mM K+, with maximum response of 94% and EC50 of 47 μM, P < 0.05).

Conclusions: These results suggest that resveratrol induced endothelium-independent vasorelaxation of HUV. The glibenclamide-, 4-AP, TEA- and BaCl2-sensitive K+ channels are involved in vasodilatory effect. However, it seems that resveratrol has additional K+ channel independent mechanism of action.

Funding: Our work has been supported by Scientific Research Grant (TR31020) from the Ministry of Science (Serbia).
ullin level 10.5% (95% CI: 2.5–17.8%) lower than the non-carriers (beta = –0.115, P = 0.011). Haplotypic analysis revealed a similar significant association with plasma adrenomedullin (overall P = 0.040).

**Conclusions:** Plasma adrenomedullin is related to IL-6 but not CRP, which is consistent with the ability of adrenomedullin to stimulate IL-6 production *in vitro*. Plasma adrenomedullin is also influenced by a common polymorphism, which means that including the genotype may improve cardiovascular risk prediction. Acknowledgement: This study was funded by Hong Kong Research Grant Council grants (HKU7229/01M and HKU7626/07M) and the Sun Chich Yeh Heart Foundation.

**P147**

**EFFECT OF CHOLINE DEFICIENT DIET ON MYOCARDIAL PERFORMANCE: THE ROLE OF CARNITINE**

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**Introduction:** Choline is considered an essential nutrient and choline deficient diet (CDD) seems to impair the heart performance. Carnitine, roughly similar in structure to choline, is a cofactor required for mitochondrial fatty acid beta-oxidation, essential for the proper function of the heart muscle. The present study explores the effects of choline deficiency on the heart and the possible protective role of carnitine.

**Materials and Methods:** Fifty Wistar Albino rats (about 2 months old) were divided in four groups? (i) control (C), (ii) rats fed with CDD (CDD), (iii) rats fed with balanced diet and carnitine in drinking water 0.15% (CARN), (iv) rats fed with CDD and carnitine in drinking water 0.15% (CDD + CARN). After 4 weeks of treatment, cardiac function was assessed using isometric conditions in the Langendorff preparations (Left Ventricular Developed Pressure: LVDP, Positive and Negative first derivative of LVDP: PDPD and N.PDPD were evaluated respectively). Histopathological evaluation of the heart specimens has been done using the Hematoxyline-Eosine and Masson counterstain.

**Results:** LVDP was 102.14 mmHg in CDD hearts vs. 122.5 mmHg in C hearts while it was 121.33 mmHg in CDD + CARN hearts (P = 0.01 vs. CDD). A statistically significant difference was also observed in N.PDPD between C and CDD hearts (2311.0) and CDD hearts (1911.42), (P = 0.026 vs. C) as well as between CDD and C + CARN hearts (2250.0), (P = 0.09 vs. CDD). PDPD was 3948.57 in CDD hearts vs. 4216.66 in C hearts and 4050.0 in CDD + CARN hearts (P = ns). Histopathology sections of CDD showed an inflammatory cellular infiltration mainly with mononuclear (lymphocytes) at the valves and myocardium which was inhibited markedly by carnitine.

**Conclusions:** Carnitine administration restores contractility of myocardial and diastolic dysfunction of the heart caused by choline deficient diet, moreover it suppresses the induced inflammatory response which needs further investigation.

**P148**

**RELAXATION OF THE VENOUS GRAFT INDUCED BY NICORANDIL**

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**Introduction:** The drug nicorandil is a vasodilator approved for treatment of angina. In addition to its well-known effect on the opening of ATP-sensitive K+ (KATP) channels, nicorandil-induced vasorelaxation also involves the opening of Ca2+-activated K+ channels. The aim of this study was to investigate the effects of nicorandil on the isolated human saphenous vein (HSV) and to define the contribution of different K+ channel subtypes in endothelium-independent nicorandil action on this blood vessel.

**Methods:** The HSV segments were collected from male patients who were undergoing coronary artery bypass surgery and studied in organ bath. HSV rings were pre-contracted with phenylephrine (10 mM). Endothelium was removed mechanically.

**Results:** Our results show that nicorandil (0.001–300 μM) induced a concentration-dependent relaxation of HSV rings pre-contracted by phenylephrine. Glibenclamide (10 μM), a selective KATP channels inhibitor, partly antagonized the relaxation of HSV. A non-selective inhibitor of K+ channels, tetracythlammonium (TEA, 1 mM), as well as charybotoxin (10 nM), an inhibitor of large conductance Ca2+-sensitive K+ (BKCa) channels and voltage-gated K+ (KV) channels, did not affect the nicorandil-induced relaxation of HSV. A non-selective blocker of KV channels, 4-aminopyridine (4-AP, 3 mM), completely abolished the nicorandil-induced relaxation of HSV, whereas margatoxin (10 nM), a potent inhibitor of KV1.3 channels, did not significantly modify the nicorandil-induced relaxation of HSV.

**Conclusions:** Our results showed that nicorandil potently relaxed HSV rings without endothelium. It seems that KATP and 4-AP-sensitive K+ channels located in the smooth muscle of HSV mediated this relaxation. The study was supported by a Scientific Research Grant from Ministry of Science and Technology Serbia, Tianjin Municipal Science and Technology Commission (09ZCZDF04200), Ministry of Science and Technology China (2009DFB30560 & 2010CB529502) and Hong Kong RGC GRF grants (CUHK4651/07M; CUHK4789/09M).

**P149**

**INVESTIGATION OF THE ANTIFIBRILLATORY DRUG INTERACTIONS BETWEEN AMIODARONE AND PROPRANOLOL IN PERFUSED RABBIT HEARTS**

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In view of the reliability of the serial-shock method of measuring ventricular fibrillation threshold (VFT) in assessing antifibrillatory potency of many antiarrhythmic drugs (Almotrefi et al. Br.J.Pharmacol 1981; 73 373–377) and the alarming reports of the proarrhythmic effects of several antiarrhythmic agents, we decided to use the above technique to study interactions that may occur when antiarrhythmic and antihypertensive drugs from different classes are combined. In the last few years, we have presented the results of several of these studies on the antifibrillatory interactions between these drugs such as lidocaine, propranolol, bretylium, valsartan and captopril. In this abstract we report the antifibrillatory interactions between amiodarone and propranolol. Studies were carried out on hearts isolated from New Zealand white rabbits of either sex weighing 1.5–2 kg. The method used has been given in details previously (Almotrefi et al. Br.J.Pharmac 1980; 71 635–639). Perfusion with either amiodarone or propranolol produced significant, dose-dependent increase in VFT. In addition, there was no significant difference in the increase in VFT produced by the combined infusion of 1 μmol of amiodarone and 0.34 μmol of propranolol and the summation of the increases produced by the separate infusion of these two concentrations. This is in contrast to a significant synergistic antifibrillatory effect of the combined infusion of lidocaine and propranolol reported previously (Almotrefi et al. Br.J.Pharmac 1999; 128 55P). The possible relevance of these results to their combined use in the treatment of cardiac arrhythmias and hypertension is discussed.
P150
THE EFFECT OF ATORVASTATIN ON CARDIAC REMODELLING

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The effect of atorvastatin on cardiac remodeling, function, and homodynamic parameters in isoproterenol (ISO)-induced heart failure were evaluated in the present study. A subcutaneous injection of ISO (5 mg/kg/day) for 10 days was used for the induction of heart failure. ISO administration produced intensive myocardial necrosis and fibrosis with a significant decrease in the arterial pressure indices, heart rate, contractility (LVEDP/dtmax) and relaxation (LVEDP/dtmin), but an increase in the left ventricular end-diastolic pressure. Rats were randomly assigned to control, treatment with only atorvastatin, and treatment with atorvastatin plus coenzyme Q10. Histopathological analysis showed a marked attenuation of myocyte necrosis and interstitial fibrosis in all atorvastatin treated groups (P < 0.001). A low dose of atorvastatin (5 mg/kg/day) significantly improved the left ventricular (LV) systolic pressure, contractility and relaxation (P < 0.01). On the contrary, a high dose of atorvastatin (20 mg/kg/day) worsened the ISO-induced left ventricular dysfunction by a further reduction of LVEDP/dtmax from +2780 ± 94 to +1588 ± 248 (mmHg/s; P < 0.01) and LVEDP/dtmin from -2007 ± 190 to -2939 ± 291 (mmHg/s; P < 0.05). Co-administration of coenzyme Q10 with atorvastatin reversed the hemodynamic depression and the LV dysfunction to a high level (P < 0.001). There was a lower level of LVEDPs in the atorvastatin+coenzyme Q10 treated groups (3 ± 1 and 4 ± 1.4 vs. 8 ± 3.5 and 14 ± 3.6 mmHg, respectively), thereby suggesting improvement in the myocardial stiffness by the combined coenzyme Q10 and atorvastatin treatment. The atorvastatin therapy attenuated myocardial necrosis and fibrosis in ISO-induced heart failure. However, a high dose of the drug considerably worsened the LV dysfunction and hemodynamic depression, which was reversed by coenzyme Q10 co-administration.

P152
SYNCHRONISED ECG-SIGNAL SUPERPOSITION: A POWERFUL TOOL TO SCREEN FOR REPOLARISATION CHANGES

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Background: Manual analysis of electrocardiographic QT/QTc-interval duration is the core of the regulatory evaluation of ECG-QT/QTc-safety (ICH E14).

Purpose: to present a simple graphical method to screen for relevant on-study changes in QT and T-wave morphology.

Methods: (i) To export lead-specific ECG-tracing data from the ECG-XML-files that are now standard for the reporting of ECG-data from all relevant on-study recordings, (ii) to synchronise the tracings for coinciding maxima (R-wave), (iii) to produce R-wave synchronised superimposed multiple line graphs of the several on-study ECG-signals.

Results: Already at a first glance this allows identifying (i) on-study changes in QT-duration, and (ii) on-study changes in the T-vector amplitude and angle. Whereas the first are HR-related, the second are not. E14 tunnel-vision only focused on QT/QTc fails to assess ST-T wave changes. Such ST-T wave changes might reflect myocardial ischaemia, electrolyte shifts, etc. However, on-study ST-T wave changes also appear in healthy subjects without evident pathological or drug-related causes, for instance after meals.

Conclusion: Graphical displays of R-wave synchronised ECG-tracings are a powerful tool to visualise on-study/on-treatment changes in QT-interval duration and ST-T wave morphology (amplitude and angle). There is need for more awareness that on-study medication-unrelated factors (such as meals) might cause relevant changes in ST-T wave morphology. Broader professional awareness of this phenomenon is needed to avoid that such physiological changes are erroneously seen as safety limiting ECG-findings.

P153
NOVEL PROPAFENONE ANALOGS HAVE ANTIARRHYTHMIC EFFECT

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Introduction: The aims of our study were: (i) to investigate the vasodilatory effect of propafenone and its newly developed derivatives on the isolated rat aorta rings, (ii) to evaluate the involvement of ion channels in their effect and (iii) to evaluate the antiarrhythmic properties of those compounds in a model of acomitine-induced arrhythmia of Wistar rats.

Materials and Methods: We synthesized two analogs of propafenone (5OF-PF and 5PF-PF) with modifications in the benzyl moiety designed on the basis of pre QSOR and pharmacoprophic studies. in vitro and in vivo experiments were in accordance with good laboratory practice.

Results: Propafenone, 5PF-PF and 5OF-PF relaxed the rat aorta rings with endothelium and without endothelium with comparable potency (EC50: 5.11 vs. 5.02 µM, 5.26 vs. 4.94 and 5.16 and 4.96 µM, 4-amino piridine (4-AP), a blocker of voltage dependent K+ channels, nifedipine, an blocker of large conductance Ca2+ channels and lidocaine, a blocker of Na+ channels antagonized the propafenone-, 5OF-PF- and 5OF-PF-induced relaxation of aorta with comparable potency. Acomitine-induced tachycardia were strongly inhibited by propafenone-, 5OF-PF- and 5OF-PF-induced relaxation of aorta with comparable potency. Acomitine-induced tachycardia were strongly inhibited by propafenone-, 5OF-PF- and 5OF-PF-containing maxima (R-wave), (iii) to produce R-wave synchronised superimposed multiple line graphs of the several on-study ECG-signals.

Conclusion: Graphical displays of R-wave synchronised ECG-tracings are a powerful tool to visualise on-study/on-treatment changes in QT-interval duration and ST-T wave morphology (amplitude and angle). There is need for more awareness that on-study medication-unrelated factors (such as meals) might cause relevant changes in ST-T wave morphology. Broader professional awareness of this phenomenon is needed to avoid that such physiological changes are erroneously seen as safety limiting ECG-findings.

P155
CO-EXISTENCE OF FATTY ACIDS CHANGES IN AORTA ARTERY AND ADIPOSE TISSUE; COMPARISON BETWEEN CAD AND NON CAD PATIENTS

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Abstract

Introduction: This study was aimed to evaluate composition and possible co-existence of change in fatty acids of aorta artery and adipose tissue in two groups of patients with different degree of atherosclerosis.

Methods: Twenty one angiographically documented coronary artery diseases (CAD) patients, and the same numbers of age, sex and body mass index -matched angiographically documented non CAD patients enrolled in this study. They were operated electively for coronary artery bypass grafting (CABG) or aortic valve replacement surgery (AVR), respectively. Small segments of ascending aorta artery and adipose tissue were dissected form the two groups during open heart surgery and subjected to fatty acid analysis.

Results: The results showed that in the CAD group, amounts of saturated and o6 fatty acids were higher, while the percent of monounsaturated and o3 fatty acids were lower than the non CAD patients for both aorta artery and adipose tissue samples. A moderate correlation was seen between amounts of fatty acids in adipose tissue and aorta artery.

Conclusion: As there are many reports which show that adipose tissue can only be used as a suitable indicator of dietary intake of exogenous fatty acids (e.g. polyunsaturated and trans fatty acids), our study suggests that modification of fatty acids with endogenous synthesis and metabolism (e.g. saturated and monounsaturated fatty acids) which were observed in both adipose tissue and aorta artery of CAD patients, may be produced during atherogenesis.
sirgunenly their mechanism of action on the vascular smooth muscle and their antiarrhythmic effect.

P154
INCREASE OF BLOOD PRESSURE AND PULSE RATE INDUCED BY CAFFEINE ARE INHIBITED BY (−)-EPIGALLO-CATECHIN-3-O-GALLATE: INVOLVEMENT OF CATECHOLAMINES
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In the previous experiment, EGCG reduced caffeine-induced locomotor activity and stereotyped behaviours, and inhibited caffeine-induced neuronal stimulant activity. This research was performed to give additional evidence that (−)-epigallocatechin-3-O-gallate (EGCG) counteracts caffeine-induced stimulant effects in animals. EGCG inhibited caffeine-induced cardiovascular activation, such as blood pressure and pulse rate. In addition, the increases of epinephrine and norepinephrine induced by caffeine, were reduced by EGCG in the blood. We suggest that EGCG may reduce caffeine-induced blood pressure and pulse rate, decreasing catecholamines levels in the blood. Therefore, EGCG counteracts caffeine-induced cardiovascular activity. The stimulant effects of caffeine should be reduced by the amount of EGCG in the green tea.

P155
HISTOLOGICAL INVESTIGATION OF THE EFFECT OF L-CARNITINE ON RAT AORTA
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Introduction: L-Carnitine is involved in the transport of long-chain free fatty acids into the mitochondria for the production of energy. It is used as nutritional supplement in myopathy, neuropathy and heart disease. The purpose of this study is to investigate the effect of l-carnitine on the aorta of aged rats.

Materials and Methods: Two groups of Wistar rats, 20 months old, were used: the study group and the control group. The study group received l-carnitine at a dose of 300 mg/kg b.w./day intraperitoneally for 35 days. The control group received normal saline in the same way. After euthanasia, specimens of the aorta were prepared for immunohistochemical study, using a monoclonal anti-insulin antibody.

Results: In the control group, the endothelial cells of the aorta showed no staining for insulin receptors. In the study group, insulin receptors were detected on endothelial cells of the aorta.

Conclusion: L-carnitine induces the expression of insulin receptors on the surface of endothelial cells of rat aorta. This result is consistent with its beneficial effect in insulin resistant diabetes, and the increase in hydrocarbon metabolism in patients receiving L-carnitine.

P156
EXPRESSION OF INSULIN RECEPTORS ON THE ENDOTHELIUM OF RAT AORTA AFTER ADMINISTRATION OF L-CARNITINE
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Introduction: L-Carnitine is involved in the removal of short and medium-chain fatty acids and the transportation of long chain fatty acids from cytosol into the mitochondria. The aim of this study was to investigate the expression of insulin receptors on endothelial cells of the rat aorta after administration of l-carnitine in old aged rats.

Materials and Methods: Two groups of Wistar rats, 20 months old, were used: the study group and the control group. The study group received L-carnitine at a dose of 300 mg/kg b.w./day intraperitoneally for 35 days. The control group received normal saline in the same way. After euthanasia, specimens of the aorta were prepared for immunohistochemical study, using a monoclonal anti-insulin antibody.

Results: In the control group, the endothelial cells of the aorta showed no staining for insulin receptors. In the study group, insulin receptors were detected on endothelial cells of the aorta.

Conclusion: L-carnitine induces the expression of insulin receptors on the surface of endothelial cells of rat aorta. This result is consistent with its beneficial effect in insulin resistant diabetes, and the increase in hydrocarbon metabolism in patients receiving L-carnitine.

P157
VARIATION IN THE CYP2D6 GENOTYPE IS NOT ASSOCIATED WITH CARVEDILOL DOSE CHANGES IN PATIENTS WITH HF
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Carvedilol is the standard of care for heart failure (HF) patients. Carvedilol is partially metabolized by the highly polymorphic enzyme, CYP2D6. To reach an effective dose while avoiding adverse drug reactions (ADR), testing of CYP2D6 genotype prior to carvedilol initiation should be considered. The objectives were to determine CYP2D6 metabolic genotypes in an Israeli cohort of HF patients and investigate the relationship between genotype, carvedilol dose, and number of ADRs in order to consider CYP2D6 genotyping prior to treatment initiation.

Descriptive and inference statistics were performed followed by correlation and regression analyses. Ninety-three Patients on carvedilol were CYP2D6 genotyped and classified as poor, intermediate, extensive, or ultra-rapid metabolizers. UM, n = 6, 6.5%; IM n = 11, 11.8%; EM n = 70, 76.3%; PM n = 5, 5.4%. The initial carvedilol dose increased significantly according to patients clinical needs in each of the four genotype groups. Twenty-two patients experienced adverse events (ADRs). There were not significant differences among the dose and the number ADRs after 3, 12 and 60 months following initiation of carvedilol treatment in each genotype group. There were no statistically significant differences in carvedilol doses among those treated with 4 medications compared with those treated with five or more concomitant drugs. Twenty-two patients were also treated with 2D6 inhibitors (amiodarone, n = 19; antidepressants, n = 4). Regression analyses revealed that genotype group affiliation and number of adverse drug reactions were not predictive of carvedilol dose changes. Patient weight was the only significant predictors for the carvedilol dose.

Conclusions: No relationship was found between carvedilol dose and patient CYP2D6 genotype and number of adverse drug reactions in an
Israeli cohort. Therefore, the recommendation of CYP2D6 genotyping prior to carvedilol initiation should be questioned.

P158
THE INFLUENCE OF RAMIPRIL ON THE INFLAMMATION MARKERS AND THE IMMEDIATE PROGNOSIS OF MYOCARDIAL INFARCTION
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The aim of the research was to find out the advantages of amprilan (ramipril) in the influence on the inflammation markers and the terminal point (death, recurrent myocardial infarction, stroke) within 3 months after the myocardial infarction. Sixty patients of 49–76 years of age with the diagnosis of acute coronary syndrome were included into the research. The concentration of C-reactive protein (C-RP), total cholesterol, triglycerides, cholesterol of high-density lipoproteins and cholesterol of low-density lipoproteins was determined in all the patients during the first 24 h and 3 months later, with echocardiography being carried out as well. Amprilan was administered starting from the first 24 h of the anginous attack emergence, and its dose titration was from 2.5 up to 10 mg/a day. The amprilan therapy reduced the frequency of angina pectoris emergence in the post infarction period (65% against 36%), brought about the deceleration of the hypertrophy intensity growth as well as the mass of the myocardial left ventricle, and reduction of C-RP concentration in the blood (by 87.6% against 36.4%). In the amprilan group the systolic blood pressure was reduced by 13.2%, diastolic blood pressure – by 9.7%, and 62% of patients reached the designated blood pressure. In the amprilan group the level of total cholesterol was reduced by 8.1% against 4.9%, of triglycerides by 10.4% against 7.3% and cholesterol of low-density lipoproteins by 7.8% against 9.8%, while the cholesterol of high-density lipoproteins content raised by 8.4% against 2.9% in the control group accordingly. No recurrent cases of myocardial infarction and stroke were registered in the amprilan group. Thus, amprilan reduced the risk of fatal cardiac and cerebral-vascular events development.

P159
THE EFFECTS OF CIPROFLOXACIN ON INFLAMMATORY MARKERS IN PATIENTS WITH ACUTE CORONARY SYNDROME
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The role of inflammation and possible vascular infection in development, progression and complications of atherosclerosis has been investigated in a large number of clinical trials. At the same time, increased concentrations of inflammatory markers in patients with acute coronary syndrome (ACS) were observed. Inflammatory markers actively participate in atherothrombotic process and can predict adverse outcomes. The aim of our study was to investigate whether antibiotic therapy initiated early (during first 48 h) in patients with ACS results in change of several inflammatory markers (serum fibrinogen, high sensitive CRP and serum amyloid A level) during 1-year follow up period. Patients with ACS were prospectively randomized into two groups: group A were given ciprofloxacin (500 mg b.d., 10 days) and group C (control; routine therapy). The inflammatory markers were determined immediately after admission and after 3, 6 months and at the end of the year. During follow-up period, patients also were reevaluated clinically for cardiovascular adverse events. At admission, the serum concentrations of inflammatory markers were increased, but there were no statistically significant difference between groups. Ciprofloxacin produced statistically significant decrease in serum concentrations of fibrinogen, hsCRP and SAA up to 1 year after the drug administration. However, despite the decrease in inflammatory markers concentrations, ciprofloxacin did not significantly reduce the rate of cardiovascular events compared with control (P > 0.05). In conclusion, the short course of ciprofloxacin immediately after ACS seems to produce a long-term decrease in inflammatory markers but such an effect does not reach clinical significance.
P160
VALIDATION OF ‘FIESOLE MISURATA’ DATABASE: AN ONGOING PROJECT TO INVESTIGATE HOW TO ENHANCE ADHERENCE TO ANTIHYPERTENSIVE MEDICATIONS (AM)

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Introduction: Non-adherence to AM is a major concern in the elderly. Indeed, up to 50% of AM recipients discontinued therapy during the first 6 months.

Methods: The aim of the project ‘Fiesole Misurata’ was to investigate the possible strategies to reduce non-adherence to AM among older community dwellers. In the first phase an ad hoc database was assembled. A sub-cohort (n = 385) of residents of Fiesole, a small town near Florence, aged 65+, were enrolled (May, 10–July, 15, 2010), and examined pharmacotherapy, co-morbidity, disability, cognitive status, nutritional and smoking habits. For all elderly residents of Fiesole (n = 2228) reimbursed prescriptions (ATC codes) and discharge diagnoses (ICD9CM codes) were retrieved from the Local Health Authority claim repository (January, 2008–July, 31, 2010). All these records were merged through an encrypted ID code. Herein, FM database was composed of a claim and a Cross-Sectional (CS) component. To evaluate the generalizability of these data, prevalence of cardiovascular diseases and medications were computed in both claim and CS cohorts. These results were compared with current medical literature.

Results: In claim dataset, annual prevalence of hospitalization for ischemic cardiomyopathy, stroke and heart failure was 1.2, 0.6, 1.4%, respectively. Concerning medications, 64.0% of participants were AM users both in claim and CS cohort; ACE inhibitors were the most used drugs (45.3% in CS, 36.5% in claim dataset) followed by beta-blockers.

Conclusions: On the basis of medical literature, ‘Fiesole Misurata’ database seems to possess valid and generalizable information in terms of cardiovascular diseases and medications.

P161
COMPARISON OF BENAZEPRIL MONOTHERAPY TO AMLODIPINE PLUS BENAZEPRIL IN THE TREATMENT OF PATIENTS WITH MILD AND MODERATE HYPERTENSION: A PARALLEL-CENTERED, RANDOMIZED, DOUBLE-BLIND, PARALLEL-CONTROLLED STUDY
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Objective: To evaluate the efficacy and tolerability of the fixed combination of amlodipine 5 mg/benazepril 10 mg once-daily therapy, compared with benazepril, 10 mg, monotherapy in patients with mild and moderate hypertension, and to evaluate the 24 h antihypertensive efficacy and the duration of action by ambulatory blood pressure monitoring.

Methods: In a multicenter, randomized, double-blind, parallel controlled trial, 356 cases of hypertensive patients after 2 weeks wash-out, and then given 4 weeks of benazepril 10 mg monotherapy, 220 patients with mean seated diastolic blood pressure (SeDBP) remained ≥90 mm Hg (1 mm Hg = 0.133 kPa) were randomly divided into benazepril 10 mg/amlopidine 5 mg (BZ10/AML5) fixed-dose combination therapy group (once a day, n = 113), and benazepril monotherapy group (daily 20 mg, n = 107). In the two groups, the patients with SeDBP ≥90 mm Hg were doubled the dosage of the initial regimen at the end of 4-week treatment for additional 4 weeks, and the patients with SeDBP <90 mm Hg remained the initial regimen for additional 4 weeks. The primary endpoint was to evaluate the improvement of SeDBP at the end of 8-week treatment. There were 74 patients (the combination therapy group n = 38, monotherapy therapy group n = 36) completed the 24 h ambulatory blood pressure monitoring which was included in the final efficacy analysis.

Results: The randomized, double-blind treatment for 8 weeks, the mean value of SeDBP reduction, the reaching target blood pressure rate and total successful response rate to the treatment (a SeDBP <90 mm Hg or a decrease of 10 mm Hg or more from baseline) were 11.7 ± 6.75 mm Hg, 65.7% and 88.5% in the combination therapy group, respectively, and were7.7 ± 6.87 mm Hg, 35.5% and 65.5% in the monotherapy group, respectively. There were statistically significant difference between the combination therapy and the monotherapy groups in all the 3 indexes (P < 0.001). The fixed combination significantly reduced systolic blood pressure (SBP) and diastolic blood pressure (DBP) values throughout the 24 h. The trough to peak ratios of DBP/SBP in the fixed compound of benazepril/amlopipine (10 mg/5 mg) and benazepril (20 mg) alone were 83.1%/76.0% and 85.8%/79.5%, respectively. Adverse events rates were 16.8% in the combination therapy group and 35.5% in the monotherapy group (P < 0.001).

Conclusions: The combination therapy with benazepril/amlopidine was superior to benazepril monotherapy and was well tolerated in patients with essential hypertension and allowing a satisfactory BP control for 24 h. This work was supported by grants from Secial Fundation by the Ministry of National Science and Technology. (2008ZX09312-018).

P162
EFFECTS OF OXIDATIVE STRESS ELICITED BY H2O2 DURING COOLING IN THE HUMAN UMBILICAL ARTERY
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Introduction: Cooling during cardiopulmonary bypass has been shown clinically and experimentally to cause hypoperfusion of the placenta and the fetus. This in vitro study was designed to assess the vasoactive effects of oxidative stress induced with H2O2 during cooling and possible mechanisms in human umbilical arteries.

Methods: With institutional approval, isolated (discarded) human umbilical strips with intact endothelium (n = 8) suspended in organ baths aerated with a gas mixture of 95% O2: 5% CO2 at resting tension at 37°C and 28°C were subjected to cumulative doses of H2O2 (10-7 M-3 M) and concentration-response curves were recorded. Then, at 28°C different strips with endothelium were incubated with L-NAME (10-4 M) (n = 8), indomethacin (10-5 M) (n = 8), or verapamil (10-6 M) (n = 8) and concentration-response curves were obtained for cumulative H2O2. The Emax and pD2 were calculated as appropriate (P < 0.05 = significant).

Results: H2O2 (10-7 M-3 × 10-2 M) elicited concentration dependent contraction in strips at 37°C (Emax = 63.5 ± 3.7, pD2 = 3.08 ± 0.0) and 28°C (Emax = 87.2 ± 3.0, pD2 = 3.7 ± 0.2). Emax and sensitivity were greater at 28°C (P < 0.05). At 28°C, compared to control, incubation with L-NAME significantly augmented (Emax = 99.7 ± 5.4) while verapamil (Emax = 37.8 ± 2.8) and indomethacin (Emax = 29.3 ± 3.7) significantly inhibited H2O2 elicited contractions (P < 0.05).
Conclusions: \( \text{H}_2\text{O}_2 \) caused concentration-dependent constriction in umbilical arteries at both temperatures. Cooling further augmented the constriction of umbilical arteries, which are under oxidative stress. Our results suggest that NOS enzyme activation, constrictor cyclooxygenase metabolites and intracellular and extracellular Ca\(^{2+} \) ions are involved in the constrictor response during cooling in human umbilical arteries under oxidative stress elicited by \( \text{H}_2\text{O}_2 \).

P164
INCREASING PREVALENCE OF HYPERTENSION AND CENTRAL OBESITY IN THE HONG KONG CARDIOVASCULAR RISK FACTOR PREVALENCE STUDY
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Introduction: Hypertension is known to be related to obesity. We studied the prevalence of hypertension over a period of 11.9 years in the population-based Hong Kong Cardiovascular Risk Factor Prevalence Study (CRISPS) cohort and investigated its association with changes in body mass index (BMI) and waist circumference.

Methods: A total of 2888, 1942 and 1798 subjects in CRISPS-1 (1995–1996), CRISPS-2 (2000–2004) and CRISPS-3 (2005–2008), respectively, were included in this analysis. Hypertension was defined as blood pressure \( \geq 140/90 \) mmHg or taking anti-hypertensive medication. General obesity was defined as BMI \( \geq 27.5 \) kg/m\(^2 \) and central obesity was defined as waist circumference \( \geq 90 \) cm in men or \( \geq 80 \) cm in women.

Results: The prevalence of hypertension increased from 18.1% to 39.4% (P < 0.001 after adjusting for age and sex). The prevalence of central obesity increased from 25.4% to 41.4%, but that of general obesity decreased from 16.8% to 14.8% (both P < 0.001 after adjusting for age and sex). Among 1347 subjects who did not take any anti-hypertensive medication at both CRISPS-1 and CRISPS-3, the change in waist circumference, but not that in BMI, was associated with the changes in both systolic and diastolic blood pressures (beta = 0.087, P = 0.015 and beta = 0.122, P < 0.001 respectively).

Conclusions: An increase in the prevalence of hypertension was observed in the cohort, which might be explained by the increase in central rather than general obesity. Our findings further confirm the importance of measuring the waist circumference, as opposed to just calculating the BMI, in this population.

Acknowledgement: This study was funded by Hong Kong Research Grant Council grants (HKU7229/01M and HKU7626/07M) and the Sun Chieh Yeh Heart Foundation.

P165
EFFECT OF VALSARTAN, ENALAPRIL, AND ALISKIREN THERAPY ON PLASMA RENIN ACTIVITY AMONG PATIENTS WITH HYPERTENSION AND CHRONIC KID
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The results of study in recent decades demonstrate the importance of activation of RAAS in the development and progression of hypertension and target organ damage, as well as the development of nephropathy, heart and vascular remodeling.

The aim was to study the changes in plasma renin during the therapy by valsartan 160 mg, enalapril 40 mg or aliskiren 300 mg in combination with a prolonged form of indapamide among patients with II-III hypertension and chronic kidney disease.

Materials and Methods: The study comprised 62 patients (24 women, 38 men) aged from 45 to 70 years with hypertension II-III degree, and CKD. Patients were randomized into groups taking: valsartan 160 mg and indapamide 1.5 mg, enalapril 40 mg and indapamide 1.5 mg, aliskiren 300 mg and indapamide 1.5 mg. The time of the investigation made up 20 weeks. Plasma renin activity (PRA) was determined by radioimmunoassay.

Results and Colloquium: In the group of patients treated by valsartan 160 mg and indapamide 1.5 mg, the level of PRA increased by 122.9%. In the group of patients treated by enalapril 40 mg and indapamide 1.5 mg increase in PRA was 109.5%. And in the group treated by aliskiren 300 mg and indapamide 1.5 mg level of PRA decreased by 56.4%. The decrease in blood pressure in all groups was similar.

Conclusion: The treatment by enalapril, valsartan and aliskirenom (among patients with hypertension II-III degree of CKD) has an equal hypotensive effect. At the same time aliskiren therapy reduces plasma renin activity. Aliskiren, blocking the RAAS at the starting point, lowers PRA, thereby reducing the progression of hypertension, chronic kidney disease and the development of cardiovascular end points.

P166
WHAT IS THE BEST WAY TO MEASURE PROTEINURIA IN CLINICAL TRIALS OF ANTIPROTEINURIC DRUGS?
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Introduction: Several studies show that protein:creatinine ratio (PCR) or albumin:creatinine ratio (ACR) from a spot urine sample is a suitable replacement for 24 h urine protein excretion in the context of screening for proteinuria. Also, PCR and ACR are as effective as 24 h urine samples at predicting patient outcomes, and potentially better at predicting renal disease progression. This study assessed whether it may be appropriate to use PCR as an alternative to 24 h urine protein excretion in clinical drug trials with endothelin receptor antagonists.

Methods: Data were gathered in a randomised, double-blind, placebo controlled study of the ETA-selective endothelin antagonist sitaxsentan 100 mg and active control in 27 patients with stable chronic kidney disease (Dhaun et al, Hypertension 2011; in press). We compared baseline 24 h urine protein and PCR measurements in terms of their correlation, agreement and variability. We then looked at the effect of treatment on proteinuria as measured by each assay, including the correlation between proteinuria at baseline and at the end of treatment.

Results: Pre-treatment, 24 h urine protein and PCR correlate well (r = 0.932, r = 0.922 and r = 0.915 on day 1, 2 and 3 respectively), indicating a strong linear relationship between the two assays. Transforming the PCR data by a factor of 100 (a PCR of 100 mg/mmol is roughly equivalent to a 24 h urine protein of 1 g/24 h) allowed direct comparison of their agreement by the Bland Altman method. Although 95% of the differences lie between the mean ± 2 SD (-1.20 to 2.24 g/day), differences within this range are clinically significant highlighting a lack of agreement between the methods. This was supported by a two-factor within subjects ANOVA which identified the assay and not the time point as a significant source of variation (P < 0.01). At baseline, the average coefficient of variation was lower for PCR than 24 h urine protein (16.8% vs 20.0%) but the range was greater (3.1–43.9% vs 3.1–35.2% excluding outliers). After 6 weeks on sitaxsentan, the average coefficient of variation was lower for PCR than 24 h protein (18.8% vs 20.8%) but the range was greater (3.1–43.9% vs 3.1–35.2% excluding outliers).

Conclusions: The average of 3 PCR measurements on consecutive days would have been no less reliable and much more convenient than the average of three consecutive 24 h urine proteins in monitoring changes in proteinuria with an endothelin receptor antagonist.
P167
A COMPARATIVE ANALYSIS OF LERCANIDIPINE AND AMLODIPINE IN THE MANAGEMENT OF HYPERTENSION
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Introduction and Objectives: Dihydropyridine calcium channel blockers (CCBs) are extensively used in the management of hypertension. However, use is limited by adverse drug reactions such as peripheral oedema. The purpose of this study is to compare the efficacy and tolerability of lercanidipine and amlodipine in patient with mild to moderate hypertension in the real life setting of blood pressure clinic.

Design and method: This was an observational study of prospectively collected data in 1277 patients attending the Glasgow Blood Pressure Clinic at Western Infirmary from May 1998 to April 2009. Treatment persistence was estimated by calculating the number of days between first prescription and last visit or discontinuation dates. The efficacy was analysed by monitoring the BP immediately before and at least 4 weeks after the introduction of each drug but before another antihypertensive agent was added.

Results: Demographic characteristics, blood pressure records and co-morbidities of 186 lercanidipine and 1091 amlodipine users were analysed. In the lercanidipine group, 153 patients had a previous history of CCBs discontinuation due to side effects or lack of efficacy. Out of these 153 patients, 113 (74%) patients persisted on lercanidipine. More than 77% of those who had stopped amlodipine were able to tolerate lercanidipine. Median duration of treatment on lercanidipine in this cohort was 413 days (interquartile range, 154–882) vs. 188 days (interquartile range, 91–497) on previous CCBs. In the whole population, both drugs exhibited a statistically significant reduction of systolic and diastolic blood pressure: 6.7/2.5 mm Hg for lercanidipine vs. 6.2/2.8 mm Hg for amlodipine. Adequate blood pressure control (≤140/85 mm Hg) was achieved in nearly 38% of patients in each group.

Conclusion: Lercanidipine and amlodipine have similar efficacy. For those who develop adverse effects as a result of using other CCBs, lercanidipine is better tolerated and is, therefore, an effective alternative.

P168
LACK OF AGREEMENT BETWEEN OSCILLOMERIC BLOOD PRESSURE FROM THE UPPERARM AND WRIST
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Background: Oscillometric methods are well established for the noninvasive measurement of blood pressure (SBP/DBP) and pulse rate (PR). There is interest in validating more convenient measurements from the wrist (W) with conventional measurements from the upper-arm (A).

Purpose: To assess agreement between SBP/DBP and PR by W and A in healthy young male and female subjects.

Methods: Non simultaneous paired W- (OMRON RX-Genius) and A-measurements (BOSO-medicus UNO) from 12 subjects under conventional phase-I trial conditions with pre- and post-dose readings were compared.

Results: For the untransformed data (387 data pairs), there was obvious bias with W measuring lower SBP (A: 109.9 mmHg; bias W-A: -11.9 mmHg; CI: -13.0 to -10.9; limits of agreement [LA]: -32.2 [CI: -34.0 to -30.4] to 8.3 [CI: 6.5 to 10.1]) and lower DBP (A: 68.3 mmHg; W-A: -13.3 mmHg; CI: -14.0 to -12.6; LA: -27.0 [CI: -28.2 to -25.8] to 0.5 [CI: -0.7 to 1.7]), with less difference in PR (A: 62.2 bpm; bias W-A: -4 bpm; CI: 3.5 to 4.6; LA: -7.2 [CI: -8.2 to -6.2] to 15.2 [CI: 14.3–16.2]). The average difference was far less when comparing post-dosing changes from baseline (346 data pairs) for SBP (A: 0.2 mmHg; W-A: -0.5 mmHg; LA: -20.6 to 21.7), and PR (A: 2.4 bpm; W-A: 1.2 bpm; LA: -14.9 to 17.3).

Conclusion: There is lack of average agreement between W- and A-measurements of blood pressure; the average bias is far less when considering post-dosing changes; however the within-subject spread of the data differences is too large to consider the methods interchangeable.

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THE INFLUENCE OF THE COMPLEX THERAPY BASED ON VALSARTAN AND ALSIKIREN ON THE LEVEL OF ALDOSTERONE AMONG PATIENTS WITH HYPERTENSION
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According to modern concepts, the renin-angiotensin-aldosterone system (RAAS) plays a vital role in regulating blood pressure. Increased aldosterone level is an independent predictor of progression of hypertension and cardiovascular events.

The aim of the investigation study was following the dynamics of the level of aldosterone in the usage of the sequent drug combinations: valsartan, aliskiren and indapamide. It was used among patients with arterial hypertension of II-III degree and chronic kidney disease (CKD).

Materials and methods. During the investigation there were examined: 41 patients (22 women and 19 men) in the age of 45–70 years with II-III hypertension, and CKD. The patients were prescribed valsartan (160 mg per day) in combination with a prolonged form of indapamide (1.5 mg). Four weeks later the patients were prescribed also aliskiren 150 mg or 300 mg. The investigation period made up 20 weeks. The verification of hormone levels was determined by radioimmunoassay using reagent kits Ria-aldosterone ‘Immunootech’ (France).

Results and Colloquium: The aldosterone level, among patients that took valsartan 160 mg, fell by 17%. Those who were prescribed valsartan 160 mg and 150 mg aliskiren experienced the decrease of the aldosterone level by 19%. And in the group treated by valsartan 160 mg and 300 mg aliskiren the aldosterone level decreased by 23%. Thus, in cases when patients with hypertension and CKD additionally received aliskiren, a decrease of aldosterone level depended fully on the dose.

Conclusion: The inclusion of aliskiren to a combination of antihypertensive therapy containing valsartan, in combination with indapamide, influenced the level of aldosterone in the dose-dependent manner. This is related to patients with hypertension II-III degree. For the patients with hypertension and CKD the combinational drug therapy appears to be most effective. It block various parts of the RAAS, and finally it improves the prognosis in terms of prevention of cardiovascular complications.

P170
COMPLIANCE MEASUREMENT-GUIDED MEDICATION MANAGEMENT PROGRAMS IN HYPERTENSION: A SYSTEMATIC REVIEW
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Introduction: Whether interventions designed to correct patients’ attitude to antihypertensive medication can improve hypertension management outcomes is unclear. Direct patient compliance management (which can include measurement, feedback and counselling), may be considered and used as an active component of hypertension treatment programs. This novel approach is known as compliance measurement-guided (medication) management (CMGM). We conducted a systematic review to determine the effectiveness of CMGM programs in essential hypertension.

Methods: Data sources included MEDLINE, EMBASE, CENTRAL, hypertension meetings abstracts, and bibliographies of identified articles. Randomized controlled trials (RCT) and observational studies (OS) were conducted.

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MOLECULAR MODELING OF ANGIOTENSIN AT1 AND AT2 RECEPTORS BASED ON THE SOLVED STRUCTURE OF CHEMOKINE CXCR4 RECEPTOR

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Introduction: Angiotensin II is the main agent of the renin-angiotensin system to regulate blood pressure and hydro-electrolytic homeostasis. Its physiological actions are mediated by two subtype receptors (AT1 and AT2) which belong to the G protein coupled receptors family (GPCR), bearing seven alpha helices connected by three extracellular and three intracellular loops and an extracellular N-terminal and an intracellular C-terminal segments. To predict the structures of the AT1 and AT2 receptors, the rhodopsin has been used as a template, but recently the CXCR4 chemokine receptor structure was solved. The CXCR4 receptor belongs to the GPCR family and contains structural elements that are absent in the rhodopsin structure such as the insertion at the third extracellular loop which contains a Cys residue that makes a disulfide bond linking this loop and N-terminal site. It was our interest to perform the homology modeling of the AT1 and AT2 receptors using the CXCR4 structure as template and to evaluate those models using the Ramachandram graph plot to validate the distribution of side chains in both models based on the Ramachandram graph plot.

Methods: The homology modeling was performed in the routine Nest of Jackal package based in the alignment of GPCRs, the What was used to insert the agonist peptide, to visualize and to create the molecular coordinates and PROCHECK from PDBsum server was used to evaluate the models.

Results: The obtained data showed that the side chains distribution in the case of the AT1 receptor was: about 33.4% in the most favourd regions, 12.4% in the additional allowed regions, 2.5% in the generously allowed regions and 1.0% in the disallowed regions. The data for the AT2 receptor also revealed a high level of allowed regions and low levels in the disallowed region: 82.9% in the most favoured regions, 11.8% in the additional allowed regions, 2.9% in the generously allowed regions and 2.5% in the disallowed region.

Conclusions: It is concluded that the use of chemokine CXCR4 receptor is a good template to predict the structure of GPCRs containing structural elements that are specific to a few receptors as the angiotensin AT1 and AT2 receptors. the kinin B1 and B2 receptors and the endothelin ETA and ETB receptors. This study will be useful to better understand the structural changes of receptor activation and to the development of antagonists as treatments of hypertension and cardiovascular diseases. Supported by CAPES, CNPq and FAPESP.

P172
THE EFFECT OF ORALLY ADMINISTERED VERAPAMIL DOSES AND THEIR CORRELATION WITH CARDIOVASCULAR PARAMETERS IN HEALTHY VOLUNTEER SUBJECTS

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Verapamil is a selective calcium channel antagonist with a primarily antiarrhythmic, antiangiogenic and antihypertensive effect. This study aimed to evaluate the effects of orally administered doses of verapamil retard tablets (Verapamil retard 240 mg) on the cardiovascular parameters (P-R interval, systolic and diastolic blood pressure, heart rate) of healthy volunteers (n = 18, both genders, 20–55 years of age) as a function of time (0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12 and 24 h) and to determine the correlation of those with the concentrations of verapamil measured in blood plasma samples. The concentration assay was carried out using high-pressure liquid chromatography (HPLC). As soon as 1 h after the administration the values steady, with very little fluctuation of plasma concentrations (X±SE = 0.5–2.4 h = 58.8 ± 1.39 ng/ml; Cmax = 81.06 ± 4.97 ng/ml; Tmax = 4.63 ± 0.74 h). The retard formulation of verapamil significantly prolongs the P-R interval at all the time points observed, as compared to the initial values (P < 0.001). The maximum prolongation percentage was found to be Emax = 19.87%, while EC50 equalled 57.89 ng/ml. Because of the retard form, the linear correlation coefficient is no more than 0.39 (r² = 0.39; P < 0.05). Systolic blood pressure significantly decreased after 6 h, while diastolic pressure was already lower during the first hour, and remained that way throughout the observed period. The maximum depression percentages for systolic and diastolic pressures are as follows: Emax = −8.8%; EC50 = 62.27 ng/ml; r² = 0.40; P < 0.05, and Emax = −11.53%; EC50 = 60.43 ng/ml; r² = 0.53; P < 0.05, respectively. Heart rate was considerably reduced in the time span of 1–3 h after application; afterwards, the values are either similar to the antecedent ones, or even higher, as some individuals may develop reflex tachycardia. Emax = 6.37%; EC50 = 67.43 ng/ml; r² = 0.096; P > 0.05.

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EVALUATION OF CARDIOVASCULAR EFFECTS OF INTRAVENOUS VERAPAMIL IN NORMAL SUBJECTS

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The aim of this study, which involved eight healthy volunteer subjects (both genders, 26–45 years of age), was to evaluate the effect of intravenous doses of verapamil (5 mg) on cardiovascular parameters, such as P-R interval, systolic and diastolic blood pressure and heart rate, and to present it as a function of time (0; 2; 7; 12; 27; 57; 117; 237 and 577 min). The concentration of verapamil was determined by high pressure liquid chromatography (HPLC). As soon as 2 min past the completion of the drug administration P-R interval is prolonged to a statistically significant extent, this effect being most prominent at the 12-min mark and lasting for 4 h (X±SE = 19.37 ± 2.62). The maximum effect (Emax) was calculated to be 23.13%. Plasma concentrations of approximately 33 ng/ml were found to be sufficient to attain 50% of the maximum effect (EC50). A positive relationship between concentration and response is established after 7 min (r² = 0.88; P < 0.001). Verapamil affects the systolic blood pressure by lowering it by up to a maximum of 7.3% (EC50 = 87.21 ng/ml). Diastolic blood pressure was considerably decreased throughout the first 30 min (Emax = −7.43%; EC50 = 35.49 ng/ml). As far as heart rate is concerned, an ample interindividual difference is exhibited. Most volunteer subject developed a reflex tachycardia, which was most salient 2–7 min after the drug had been administered. In that interval, heart rate was about 12% higher, on average, than prior to administration.
P174
PROTECTIVE EFFECTS OF RESVERATROL ON CARDIAC AND VASCULAR TISSUES FROM RENAL HYPERTENSIVE RATS
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Introduction: Hypertension is responsible for many cardiovascular damages, including cardiac hypertrophy and oxidative stress. Increased oxygen reactive species (ROS) levels are known to induce cardiac hypertrophy and to impair the nitric oxide (NO)-dependent vasorelaxation. Several epidemiological studies and clinical trials have demonstrated beneficial effects from treatment with antioxidant substances as co-adjuvant drugs to the classic anti-hypertensive therapy. The red wine derived antioxidant agent Resveratrol (Resv) has cardioprotective properties. Since the renovascular hypertension induced by the two kidneys-one clip (2K-1C) model (Goldblatt surgery) increases the ROS generation, it becomes suitable to investigate the Resv properties on cardiovascular apparatus. The present study was aimed to evaluate the effects of the treatment with Resv on the Hypertrophy Cardiac Index (HCI) and the aortic ROS levels from 2K-1C rats.

Methods: Renovascular hypertension was induced in male Wistar rats (180 g) by the implantation of a silver clip (0.2 mm opening) in the renal artery (2K-1C). Control rats underwent to abdominal laparatomy (two kidneys, 2K). Resv treatment (20 mg/kg, gavage) began 1 day after surgery and was performed three times a week, during 6 weeks. Wet hearts were weighted and the cardiac hypertrophy was measured in function of kidneys, 2K). Resv treatment (20 mg/kg, gavage) began 1 day after surgery and was performed three times a week, during 6 weeks. Control rats underwent to abdominal laparatomy (two kidneys, 2K). Results: Treatment with Resv on the Hypertrophy Cardiac Index (HCI) and the antioxidant agent Resveratrol (Resv) has cardioprotective properties. Since the renovascular hypertension induced by the two kidneys-one clip (2K-1C) model (Goldblatt surgery) increases the ROS generation, it becomes suitable to investigate the Resv properties on cardiovascular apparatus. The present study was aimed to evaluate the effects of the treatment with Resv on the Hypertrophy Cardiac Index (HCI) and the aortic ROS levels from 2K-1C rats.

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Results: HCl was significantly higher in 2R-1C rats (4.09 ± 0.23 mg/g) when compared to 2K rats (2.80 ± 0.13 mg/g) (n = 8, P < 0.001). Resv treatment did not alter the HCl in 2K rats (2.73 ± 0.13 mg/g), but restored it in 2R-1C rats (3.14 ± 0.17 mg/g) (n = 8, P < 0.001). Basal DHE-fluorescence values of the endothelium (F0 = 35.70 ± 4.82 U) or the smooth muscle (F0 = 15.30 ± 2.39 U) from 2K-1C rats were significantly higher than the values obtained in the endothelium (F0 = 5.13 ± 1.53 U) or the smooth muscle (F0 = 12.24 ± 1.89 U) from 2K rats (n = 4, P < 0.001). Resv treatment did not alter the basal DHE-fluorescence values of the endothelium (F0 = 4.13 ± 0.16 U) or the smooth muscle (F0 = 15.30 ± 2.39 U) from 2K rats but restored the endothelial (F0 = 7.09 ± 2.03 U) and muscular (F0 = 28.36 ± 8.68 U) values from 2R-1C rats (n = 4, P < 0.001).

Conclusions: Renovascular hypertension increased the HCl and the vascular ROS levels, which were inhibited by Resv treatment. These beneficial effects from the antioxidant therapy with Resv may improve the cardiovascular functions. Financial Support: UNAERP.

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INCREASED RESPONSIVENESS TO BRADYKININ IN THE TRANSGENIC RAT AORTA OVEREXPRESSING B1 RECEPTOR EXCLUSIVELY IN THE ENDOTHELIUM
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Introduction: Bradykinin (BK), a short-lived effector is mediated by B2 receptors (B2R) and participates in the acute phase of the pain and inflammatory responses whereas Des-Ang9-BK (DBK) mediated by B1 receptors (B1R) in the chronic phase of the responses. The most prominent tissue, in which B1 receptors are induced under inflammatory conditions, is the endothelium of vessels. An increased susceptibility to endotoxic shock was shown in rats overexpressing the B1R [TGR (Tie2B1)] exclusively in the endothelial cells.

Methods: Vectorial responsiveness of the kinins was investigated in this transgenic rat. Concentration-relaxation curves for BK and DBK were obtained in thoracic aorta rings pre-contracted with nor-epinephrine and the vascular reactivity determined.

Results: The potency and the maximal response were markedly enhanced in the responses to DBK but BK-induced effect was also potentiated in TGR (Tie2B1) compared to the control animal. It was found that the expression levels of B2R (three folds) in addition to the B1R (seven folds) increased in the transgenic animal which explained the increased reactivity of the aorta to BK.

Conclusions: Our results indicate that “down regulation” of B2R did not occur in rats with endothelial overexpression of kinin B1R, in contrast to that observed with B1R in mice overexpressing the B2R in multiple tissues. Our results suggest that tissue specific fine tuning occurs between the two subtypes of kinin receptors in the regulation of the vascular responsiveness. Therefore potent and selective new B1 receptor antagonists should be developed with the potential to treat several cardiovascular diseases. Supported by FAPESP and CNPq.

P176
EVIDENCE THAT THE 3',5'-UNTRANSLATED REGION OF THE RAT ANGIOTENSIN II AT1 RECEPTOR CDNA MODULATES CELL SIGNALLING IN VASCULAR
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Introduction: The majority of well-known cardiovascular and renal effects of angiotensin II (AngII) are mediated through the AT1 receptor (AT1R). It is also known that the AT1R mediates not only hemodynamic but also inflammatory and fibrogenic functions of AngII. Vascular smooth muscle cells respond to the agonist AngII with increases in the cytosolic Ca2+ concentration and potent contractions, which are quickly desensitized. It is noteworthy that a spontaneously immortalized rabbit arterial smooth muscle cell line (RA) that contains the endogenous AT1 receptor induces specific desensitization of Ca2+ responses induced by AngII. However when these cells were transfected with AT1R (RA-AT1R) gene containing only the coding region, it was found that Ca2+ responses were also inhibited to repeated stimulations with AngII analogue [Lys2]-AngII.

Methods: In order to assess if this phenomenon was due to the overexpression of the exogenous AT1 receptor in this cell line, transfection was also performed with the AT1 receptor gene containing the coding region and the 3′, 5′-untranslated region of the receptor cDNA (RA-AT1R+3′, 5′-UTR). Binding assays performed in both cell lines expressing the RA-AT1R and in RA-AT1R+3′, 5′-UTR.

Results: The binding affinities (IC50) determined by competitive binding studies were not significantly different between that of the control cells without transfection (endogenous AT1R) or with transfection (exogenous RA-AT1R and RA-AT1R+3′, 5′-UTR). Real-time PCR was performed to determine the expression levels of the three target genes. The results showed that the endogenous rabbit AT1 receptor expression level was similar in the RA, RA-AT1R and RA-AT1R+3′, 5′-UTR cell lines, but the exogenous AT1R expression level were much higher than the endogenous receptor in the transfected cell lines.

Conclusions: This finding indicated that change in the functional expression of RA-AT1R cell line was not due to the overexpression of the exogenous AT1 receptor and provided evidence that the 3′, 5′-untranslated region in the AngII AT1R mRNA plays a role in the regulation of the Ca2+ responses to the peptides, AngII and Lys2AngII in smooth muscle cell line. Better identification of the AT1R offers exciting possibilities for new therapeutics to target the diverse actions of the angiotensin peptides. Supported by FAPESP and CNPq.
The aim of our study was to determine the effect of valsartan on the androgen status and erectile function in patients with hypertension. Sixty patients of 40–65 years of age with the diagnosis of hypertension were included into the research. The ambulatory blood pressure monitoring was determined in all the patients during the first 24 h and 3 months later. All patients answered questions of questionnaire on aging male symptoms scale and international index of erectile function before and 3 months after the course of antihypertensive therapy. Patients of the first group received angiotensin II receptor antagonist (valsartan) in monotherapy. Valsartan was administered starting from the first 24 h after destabilization of blood pressure, and its dose titration was from 80 up to 160 mg/day. Traditional treatment of hypertension with ACE inhibitors, calcium antagonists, diuretics and beta-blockers was assigned to patients of the control group. Valsartan treatment reduced the intensity of the symptoms of erectile dysfunction in men with hypertension (by 11.3% against 2.2% in the control group). This therapy has led to a decrease in symptoms of androgen deficiency (20.2% against 12.1%, respectively). Reduction in systolic (SBP) and diastolic blood pressure (DBP) were comparable in both groups. During therapy with valsartan, we noted an increase in the number of patients of category «dipper», while the number of patients remaining categories («over-dipper», «non-dipper», «night-peekers») decreased. In the control group, the trend towards normalization of jet lag is not observed. Therapy with valsartan normalizes diurnal variations in blood pressure, reduces the symptoms of androgen deficiency and didn’t contribute to erectile dysfunction.
P133
MONITORIZATION OF SURFACE WORKING AREAS CONTAMINATION BY CICLOPHOSPHAMIDE – REVIEW

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Introduction: Cancer is an increasingly common disease. Chemotherapy with antineoplastic agents (AN) plays a major role in oncologic treatments, resulting in an increasing number of AN preparations in hospitals. Cyclophosphamide (CP) is one of the most used AN and is classified as carcinogen. CP is a prodrug and its metabolites are genotoxics by interaction with DNA. To assess the risk of occupational exposure and contamination with CP studies have been made using methods that include biological, surface, and air monitoring. Therefore, the aim of our study is to review the methods used in surface monitoring.

Methods: This study is based on a bibliographical review about the methods of quantification of CP present in the surfaces of the biological safety cabinets (BSC). We have chosen CP because it can be easily detected and is a marker drug that reflects the level of exposure. Wipe samples were collected from several surfaces of the BSC and they were analyzed by gas chromatography – mass spectrometry.

Results: Previous studies have demonstrated the surface contamination in hospital environment. A UK study showed some contamination in surfaces within wards. Another Italian study revealed a large amount of CP mostly on workbenches, on the floor, on door handles and storage shelves. Two Sweden studies proved contamination on primary packaging and on the floors.

Conclusion: Wipe sampling is a good method to demonstrate surface contamination by CP. AN surface monitoring helps to reduce contamination by antineoplastic agents and promotes the correct handling, improving the risk reduction of occupational exposure.

P179
ANTIBACTERIAL ACTIVITY OF ESSENTIAL OILS AND EXTRACTS OF CULINARY AND MEDICINAL HERBS

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Due to the increasing incidence of antibiotic resistant bacteria, new antibacterial treatments are urgently needed. Throughout the ages, herbs have been used for their antiseptic actions and to preserve meat before the invention of refrigerators, although in many cases their antibacterial activity has not been studied scientifically. We have examined the antibacterial activity of the essential oils of fourteen culinary and medicinal herbs against the bacterium *Esherichia coli* DH5α. The oils were tested for their ability to inhibit the growth of *E. coli* in a disc diffusion assay, and in liquid culture, and their ability to kill *E. coli* in a zone of clearance assay. Essential oils of coriander, fennel, lavender, lemon balm, lemon grass, mandarin, peppermint, pine, rosemary, sage, tea tree, and thyme inhibited the growth of *E. coli* in a disc diffusion assay and in liquid culture, and killed *E. coli* in a zone of clearance assay, whereas grapeseed oil (used as the negative control) and neem oil did not exhibit any antibacterial properties and ylang ylang essential oil displayed only very slight antibacterial activity. Four batches of lemon balm essential oil with differing antibacterial activity were analysed by gas chromatography mass spectrometry and their chemical profiles were compared, to identify which compounds might be responsible for antibacterial activity.

P178
THE ELECTRONIC NOMOGRAM FOR DOSAGE CALCULATIONS

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Introduction: Not only is it troublesome for medical professionals to calculate drug dosages, especially those which are determined per body surface area such as anti-cancer drugs, but also mistakes in doing so may result in fatal outcomes. There is a need for a new approach to the method of calculation that reduces the effort required and prevents potential malpractice. The author has invented an electronic monogram for dosage calculations (a dosage e-nomogram) which can be used as a calculator on a smartphone or a tablet PC, with which the user is able to calculate dosages quickly, through a graphical user interface, visually, and intuitively.

Methods: The dosage e-nomogram comprises (i) a pull-down menu for selecting the drug, (ii) a pull-down menu for deciding the indicative disease, (iii) a numerical line for deciding the dose per body surface area, and (iv) a coordinate plane for calculating the dosage. On the coordinate plane, the abscissa is used for body weight and the ordinate is used for weight and height values, calculates the value of the dosage, and displays it. The user is able to adjust the location of the pointer graphic with reference to the displayed coordinates.

Results: The user is able to calculate the dosage of a drug quickly and through a graphical user interface on a dosage e-nomogram. The contours on the coordinate plane make it possible for the user to understand the results of the calculation visually and intuitively.

Conclusions: The dosage e-nomogram reduces effort required by medical professionals and prevents malpractice resulting from calculation errors. It can become an essential tool of drug prescribing in the age of smartphones and tablet PCs.


P180
OLEA EUROPAEA L. LEAF EXTRACT MODULATES COLD RESTRAINT STRESS-INDUCED OXIDATIVE CHANGES IN THE RAT LIVER AND PLASMA

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It is known that immobilization stress accelerated by cold can disrupt the oxidant/antioxidant balance and cause oxidative damage to several tissues by altering the enzymatic and non-enzymatic antioxidant status, protein oxidation and lipid peroxidation. We have recently demonstrated beneficial effects of different single doses of natural antioxidant, standardized dry olive (Olea europaæa L.) leaf extract (OLE) in a cold restraint stress (CRS)-induced gastric lesions in rats and its influence on oxidative parameters in gastric mucosa. The aim of this study was to investigate the long-term pretreatment efficacy of OLE and its potential in a modulation of CRS-induced oxidative changes at the level of liver and plasma. Experimental animals were divided into four groups: Control, OLE-treated (80 mg/kg), CRS non-treated, and CRS treated with OLE (CRS + OLE) group. Malondialdehyde (MDA) level as an index of...
lipid peroxidation, superoxide dismutase (SOD) and catalase (CAT) activities were measured spectrophotometrically in plasma and liver tissue homogenates. MDA level significantly increased, while SOD and CAT activities were significantly decreased in liver and plasma of the CRS group. Long-term supplementation with OLE provided decrease of lipid peroxidation in liver and plasma of rats exposed to CRS. Additionally, in the CRS + OLE group, the activities of two antioxidative enzymes significantly raised in comparison with the CRS group. The results obtained indicate that OLE supplementation provides oxidant/antioxidant balance in liver and plasma during stress condition. The antioxidative effect of total OLE most probably results from the ability of its phenolic constituents to scavenge reactive oxygen species which initiate lipid peroxidation.

**P181**
THE EFFECT OF PENNYROYAL EXTRACT ON BOWEL EXITABILITY IN RAT ILEUM IN INVITRO MODEL

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**Digestive Tract**  
**Abstract text:** Previous studies indicated that PENNYROYAL is a plant which is gaining decreasing acceptance as a folk remedy for treatment of the irritable bowel disease although the mechanism of this action is not clear yet.  
Male wistar rats (220–250 g) were used. Pieces of ileum (2-3 cm) were mounted in a 50 ml organ bath containing tyrode solution with temperature (37°C) and bubbled with (95%O2.5%CO2). The ileum was stimulated at 0.1 Hz and contraction was recorded by physiograph. It has been shown that 0.1 Hz stimulation of rat ileum induced contractions which are depressed by Atropin. Addition of aqueous extract of PENNYROYAL extract to the organ bath during 0.1 Hz stimulation decreased contraction in a Dose dependent manner (EC50% = 0.6 mg/ml). It seems that PENNYROYAL shows its effect by inhibition cholinergic system.

**Key words:** PENNYROYAL bowel exitability rat ileum.

**Results:** PENNYROYAL induced a concentration-dependent relaxation of SRC with EC50 of 9.52 μM and Emax of 94% and contractions provoked by oxytocin with EC50 of 21.88 μM and Emax of 95% (P < 0.05). GLB (10 μM), 4-AP (1 mM), TEA (1 mM) antagonized the response to RSV in both, oxytocin induced contractions and SRC. Relaxation achieved by concentration of 100 μM RSV was insensitive to K+-channels blockers.

**Conclusions:** RSV is uterine relaxant and can be used in tocolysis. The antagonism of RSV effect by different K+-channels blockers suggests that K+-channels are involved in reseratrol action on the contractions of rat uterus. It seems that RSV, when applied in high concentration, may exert an additional mechanism of action.

**Funding:** Our work has been supported by Scientific Research Grants TR 31020 from the Ministry of Science (Serbia).

**P183**
GCP-COMPLIANCE OF NON-COMMERCIAL CLINICAL TRIALS ON MEDICINAL PRODUCTS

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**Introduction:** The International Conference on Harmonization good clinical practice guideline (ICH-GCP-E6) from 1996 defined the standard for clinical research. This guideline was initially only adopted as a standard for commercial trials, but the implementation of GCP in national legislation after Directive 2001/20/EC required the same standards for non-commercial trials. In Austria the directive was implemented in 2005 and was followed by a decline in non-commercial trials.

**Materials:** The GCP-compliance of non-commercial protocols submitted to the ethics committee of the Medical University Vienna for the years 2003, 2007 and 2010–2013 was/will be assessed by a standardised checklist covering 60 key elements of GCP. GCP shortcomings will be summarized and analysed over the years.

**Results:** So far 26 Clinical Trial Submissions from the years 2010 and 2011 were assessed. Two protocols were found to be insufficient to apply a thorough check. Of the remaining 24 protocols, none was 100% in agreement with GCP requirements. The most common shortcomings were inappropriate statistical details: how to deal with missing/useless data (20 protocols) and protocol deviations (22). Insufficient description of Quality Control/Assurance issues were also found: Inadequate details on Monitoring (11), Audits and Inspections (18 protocols). Packaging and Labelling of the investigational medicinal product was inadequately presented in 14 protocols.

**Conclusion:** Most of the non-commercial study protocols submitted to an Ethics committee were not fully covering all GCP requirements. Problems were observed in Quality Control/Assurance, Packaging and Labelling and Statistics. Additional support and GCP training is therefore important for non-commercial Sponsors and Investigators.

**P233**
INHIBITORY EFFECT OF PHENELZINE ON HUMAN PRIMARY AMINE OXIDASE AND ITS USE AS REFERENCE FOR SCREENING OF NOVEL SSAO/VAP-1 INHIBITORS

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Human adipoctyes express almost the highest levels of semicarbazide-sensitive amine oxidase (SSAO), a cell-surface enzyme also found in vessels. SSAO, which is currently renamed primary amine oxidase, is identical to Vascular Adhesion Protein-1 (VAP-1), an adhesion molecule mainly found in endothelial cells of inflamed regions. Recently,
novel SSAO/VAP-1 inhibitors were tested and patented for their anti-inflammatory properties (Dunkel et al. Curr Med Chem. 2008 15 1827-39), while the prototypical inhibitor, semicarbazide was reported to limit fat accumulation in rodents (Mercader et al. J Obes 2011 in press). Phenelzine, a MAO-inhibitor used for the treatment of depression, has been reported to inhibit SSAO also, but such interaction was not evidenced in man. Our aim was to assess whether phenelzine and other novel chemical entities (NCEs) can inhibit SSAO in human fat cells more efficiently than semicarbazide. Pieces of human abdominal subcutaneous adipose tissue (AT) were obtained from premenopausal women undergoing plastic surgery (mean BMI: 31.7 kg/m², n = 15). Three independent approaches demonstrated that phenelzine exhibits a high affinity for the human SSAO/VAP-1. First, phenelzine was the most performing agent among 1120 compounds of the Prestwick library tested in a high-throughput screening, owing to a miniaturized method that detected, by Amplex-Red fluorescence, the hydrogen peroxide generated by human AT preparations during amine oxidation. Second, computational docking studies using the X-ray structure of human SSAO showed that phenelzine was docking to the active site of the human SSAO model. The docking orientation exhibited an ammonium group close to the catalytic oxygen atom of the topaqunone residue and to Asp 386, that was, with several other points, comparable to the previously described docking of known substrates: methylamine and benzylamine (Bonauto et al. Biochimie, 2010; 92 858–68). Third, Human SSAO activity of human AT homogenates was inhibited both by semicarbazide and phenelzine, while pargyline was without any effect. Phenelzine totally inhibited 1 mM benzylamine oxidation at 100 nM while 1 mM of of semicarbazide was necessary for complete blockade. Under the same conditions, the NCE SZV2142 exhibited interesting affinity and inhibitory capacity towards the human SSAO/VAP-1. Our results indicate that novel generation of SSAO/VAP-1 inhibitors may be successfully tested in human AT. Since several of the recent effects of such inhibitors rely with inflammation or fat accumulation, their related NCEs could more be useful than semicarbazide for the treatment of obesity.

References:
Table 1.

<table>
<thead>
<tr>
<th></th>
<th>OCT (h)</th>
<th>CTT (h)</th>
<th>WGT (h)</th>
</tr>
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<tbody>
<tr>
<td>placebo</td>
<td>3.9 ± 0.7</td>
<td>35.7 ± 9.0</td>
<td>39.5 ± 8.56</td>
</tr>
<tr>
<td>LOP</td>
<td>6.6 ± 3.31</td>
<td>70.7 ± 19.41</td>
<td>77.3 ± 19.61</td>
</tr>
<tr>
<td>LOP + MNTX</td>
<td>6.0 ± 3.21</td>
<td>65.6 ± 17.71</td>
<td>71.5 ± 17.21</td>
</tr>
</tbody>
</table>

\(^p < 0.05\), compared to placebo.

Conclusion: Subcutaneous injection of 12 mg MNTX lacks significant effects on the delay of OCT, CTT and WGT as induced by LOP.

P339

THE USE OF ACCESS TO PROTOCOLS AND RESULTS OF CLINICAL TRIALS

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Information that is readily available in the medical literature on new chemical entities (NCE) is incomplete and potentially biased. Recent cohort studies have focused on misreporting of trials within publications by comparing journal articles either with documents of the regulatory agencies or with trial protocols. A comparison of information from FDA reviews with journal articles showed that 50% of efficacy and 65% of harm outcomes per trial were incompletely reported on 33 new drug applications (NDAs) for new molecular entities approved by FDA in 2001–2002, i.e. the time when the results of clinical trials began to be available at the FDA site www.ClinicalTrials.gov. Thus, trial outcomes submitted to regulatory agencies became publicly available. Discrepancies between the trial information of FDA review report and information found in published trials consist of more favorable presentations of NDA drugs in the publications (47% unfavorable outcomes omitted). Publication bias: many trials have not been published at all 5 years after US FDA approval, what limits the number and scope of studies available for review by clinicians, but also affect the results of systematic reviews and meta-analyses. Misreporting of trials was difficult to detect without access to currently available registries and result databases. Randomized trials of modern medicine could be judged only with full transparency. Any publication that flows from the trial has to be reported without bias, misconduct, data suppression, selective reporting and misrepresentation. It is worth noting that WHO established the International Clinical Trials Registry Platform (ICTRP), with standardized entry items. In sum, there is nowadays a full disclosure worldwide of trial outcomes, with increased access to full protocols and regulatory agency submissions. However, the FDA site www.ClinicalTrials.gov does not guarantee that trial results will appear in a timely manner in the scientific literature. Therefore, the role of clinical pharmacologists became even more important. It became mandatory for clinical pharmacologists to use public availability of clinical trial results to ensure that the proper information reaches both the patients and the clinicians.
P184
THERAPEUTIC SUBSTITUTION – SWITCHING BETWEEN DRUGS IN THE SAME THERAPEUTIC CLASS
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Introduction: Drugs in the same therapeutic class are considered equivalent in terms of efficacy. Switching to another drug in the same therapeutic class in Croatia is driven by decision of the prescribing physician. The reasons could be patient issues (pharmacokinetics, adverse drug reactions) or lower drug price. Unnecessary therapeutic substitution can lead to reduced compliance and medication errors.

Patients and Methods: In a prospective study, we included 454 acutely ill elderly patients admitted to the Department of Internal Medicine, University hospital Osijek. Prescribing of beta blockers (BB), ACE inhibitors (ACEIs), calcium channel blockers (CCBs), HMG-CoA inhibitors (statins) and proton pump inhibitors (PPIs) was analyzed both at admission and at discharge.

Results: 40.3%, 45.8%, 24.0%, 24.0% and 15.0% of all admitted patients was using BBs, ACEIs, CCBs, statins and PPIs, respectively. Among BBs users, 8.7% (16/183) was substituted, with 21.4% nebeivolol users switched to another BB. Of all ACEIs users, 19.8% (46/232) was substituted, with the highest incidence in enalapril users (30.8%). Among CCBs users, 8.3% (9/109) was substituted, with the highest incidence in lacidipine users (15.2%). Of all statins users, 22.9% (25/109) was substituted, most often patients taking simvastatin (29.4%). Among PPIs users, 16.2% (11/68) was substituted, with 23.6% esomeprazole users switched to another PPI.

Conclusion: Therapeutic substitution had high incidence in all drug classes analyzed. The incidence of substitution was highest in patients taking ACE inhibitors (ACEIs) and HMG-CoA inhibitors (statins): around 20% of patients in each group was switched to another drug in the same class. It can be assumed that switching was more frequent where there were more prescribing physicians. The expectation of the prescribing physician that the new drug would have more favourable risk to benefit profile for the patient contributed to most cases of therapeutic substitution.

P185
SCREENING AND RECRUITMENT PROCEDURES OF HEALTHY VOLUNTEERS IN A PHASE I CLINICAL TRIAL UNIT: EXPERIENCE OF 38 BIOEQUIVALENCE STUDIES
Clinical Pharmacology Service, La Paz University Hospital, School of Medicine, Universidad Autónoma de Madrid, IdiPAZ, Spain

Introduction: This study reports and analyzes screening and recruitment procedures of 38 bioequivalence (BE) studies.

Material and Methods: Thirty-eight BE randomized crossover studies were conducted. The studies were all designed according to the requirements of EMA for BE studies and developed in the Phase I Clinical Trial Unit of the Pharmacology Department (School of Medicine, Universidad Autónoma de Madrid).

Sample size calculated, number of volunteers and reason for exclusion, withdrawal and dropped were recorded.

Results: For the included 38 trials, a total of 2405 healthy volunteers were informed about a BE trial and 2202 (91.6%) decided to sign the informed consent form. Of them, 233 did not return to initiation visit (10.6%). In the screening period 623 volunteers (28.3%) were not suitable for the study. The main reasons were: withdrawal of informed consent (16.4%), positive in urine abuse drug test (9.6%), hyperbilirubinemia (5.6%) and hypertransaminasemia (4.7%). Therefore a total of 1579 fulfilled inclusion criteria and 1514 were included (65 volunteers were considered as reserves). During the study, 35 volunteers (2.3%) dropped for personal reason and 17 were excluded (47% due to a positive result in urine abuse drug test). A total of 1462 volunteers were suitable for the main analysis.

Conclusion: To select 1579 valid participants in BE trials we needed to give the trial information to 2405 potential participants and to make the initiation visit in 2202 volunteers. Only a 4.1% of the healthy volunteers interviewed and fulfilling selection criteria were finally not included. Post-randomization losses were also low (3.6%).

P186
THE BIOEQUIVALENCE STUDY OF GLUCOSAMINE 1500 MG SACHET IN HEALTHY THAI MALE VOLUNTEERS
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Introduction: Glucosamine is widely used as an adjuvant treatment in osteoarthritis. Several Thai pharmaceutical companies have produced generic glucosamine. However, data supporting the efficacy of these generic products are lacking. This study aimed to investigate the bioequivalence of 1500 mg sachet of the reference (Viatriil®), Rotta Pharm, Co. Ltd.) and the test product (Kosamine®, Pharma Nueva Co. Ltd.) in 26 healthy Thai male volunteers.

Methods: This was a single dose, two treatments, two periods, two sequences crossover study with a 1-week washout period. Thousand and five hundred milligram sachet of glucosamine sulphate, either reference or test products, in 250 ml of water was administered in fasting state. Blood samples were collected at pre-dose and 1, 1.5, 2, 2.5, 3, 3.25, 3.5, 3.75, 4, 4.25, 4.5, 5, 6, 8, 10, 12, 24 & 48 hrs post-dose. Plasma glucosamine concentrations were analyzed using HPLC. Pharmacokinetic parameters were determined using noncompartmental analysis.

Results: Ninety percent CI of AU0-t and AU0-inf were 82.62–113.99% and 84.08–114.27%, respectively. Ninety percent CI of Cmax was 83.88–126.60%, which was over the limit of 80–125% but was within the acceptance limit of for high variability products of 75–133%. T1/2 and Kel of test and reference products were statistically insignificant (1.04 ± 0.78 vs 1.12 ± 0.79 h and 0.84 ± 0.31 vs 0.79 ± 0.32 1/h, respectively). Both products had similar Tmax. No serious adverse events were observed.

Conclusions: This study showed the bioequivalence of the test and reference products and suggested the high variability of Cmax in this type of glucosamine formulation in Thai population.

P187
BIOEQUIVALENCE STUDY OF A GENERIC QUETIAPINE (KETIPINOR®) IN HEALTHY VOLUNTEERS
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Introduction: Quetiapine is an atypical antipsychotic indicated for the treatment of schizophrenia and related psychoses. It has been increasingly used instead of the classical antipsychotic drugs due to its lower
extrapyramidal side effects. Use of generic drugs is essential due to economic reasons. Interchangeability of drugs is determined by bioequivalence studies. We aim to study the bioequivalence of a generic quetiapine (Ketipinor®, manufactured by the Orion Corporation, Finland) and the innovator product (Seroquel® AstraZeneca, UK).

**Methods:** The study was a randomized, two-way crossover design with a 2-week washout period in 24 healthy male volunteers. After a single 200-mg oral dosing, serial blood samples were collected at appropriate interval up to 48 h. Plasma quetiapine concentrations were determined by validated LC-MS/MS method. Pharmacokinetic parameters were estimated using the WinNonlin® software with non-compartment model analysis.

**Results:** The mean ± SD of maximum plasma concentration (C\text{max}), the area under the plasma-concentration time curve from 0 to 48 h (AUC\text{0-48}) and the area under the plasma-concentration time curve from 0 to infinity (AUC\text{0-\infty}) of Ketipinor® v.s. Seroquel® were 632.27 ± 304.43 v.s. 638.83 ± 214.49 ng/ml; 2.625.21 ± 972.14 v.s. 2.511.82 ± 704.21 ng/h/ml and 2.640.25 ± 979.10 v.s. 2.526.45 ± 704.37 ng/h/ml, respectively. The time to reach C\text{max} (T\text{max}) of Ketipinor® and Seroquel® were 1.34 ± 1.11 and 1.01 ± 0.63 h., respectively. The T\text{max} of Ketipinor® was within the acceptance range of ±20% of the median T\text{max} of Seroquel®. The 90% confidence interval of the ratios of the log-transformed data of C\text{max}, AUC\text{0-48} and AUC\text{0-\infty} were 80.75–102.60%, 91.32–108.42% and 88.47–106.77%, respectively, which were within the acceptance range of 80.00–125.00%. The power of test for C\text{max}, AUC\text{0-48} and AUC\text{0-\infty} were 92.16%, 96.34% and 95.96%, respectively.

**Conclusion:** Ketipinor®, used in this study, was bioequivalent to Seroquel® in terms of both the rate and extent of absorption under fasting condition.

**Keywords:** Bioequivalence, Ketipinor®, pharmacokinetics, quetiapine, Seroquel®.

This study was supported by the Harn Thai Pharma (2508) Co. Ltd. Bangkok, Thailand and Prince of Songkla University, Thailand.

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**P188 BIOEQUIVALENCE STUDY OF 70 MG ALENDRONATE SODIUM TABLETS IN HEALTHY THAI VOLUNTEERS**

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Siriraj Clinical Research Center, Mahidol University, Thailand

**Introduction:** Alendronate is one of the pharmacologic therapies for osteoporosis. It is an important representative of the bisphosphonates that are effective for preventing bone loss associated with estrogen deficiency, glucocorticoid treatment and immobilization. The aim of this study was to determine the bioequivalence of 70 mg dose of alendronate sodium tablets between the test product (Tevanate® 70 mg; Teva Pharmaceutical Industries Limited, Israel) and the reference product (Fosamax®; Merck Sharp & Dohme De Mexico).

**Materials and Methods:** The study was carried out with a single dose, two-treatment, two-period, two-sequence randomized crossover design under fasting condition with a minimum of 7 days washout period in 68 healthy Thai male and female volunteers. A single dose of alendronate tablet 70 mg was administered to enrolled volunteers along with 220 ml of drinking water after an overnight fasting of at least 10 h. Plasma samples for determination of alendronate were obtained pre-dose and at frequent intervals for up to 7 h post dose. Alendronate plasma concentrations were quantified by a validated method employing liquid chromatography with tandem mass spectrometry (LC-MS/MS) method with the lower limit of quantification (LLOQ) of 4 ng/ml.

**Results:** The 90% confidence interval of C\text{max}, AUC\text{0-tlast} and AUC\text{0-\infty(obs)} in this study was entirely within the equivalence criteria (80.00–125.00%) which was 85.25%-109.74% for C\text{max}, 80.80–106.69% and 82.93–107.63% for AUC\text{0-tlast} and AUC\text{0-\infty}, respectively, with the power more than 80%. In addition, no significant differences of the T\text{max} parameter between the two studied formulations were observed (P > 0.05).

**Conclusion:** Therefore, it was concluded that the two tablet formulations of alendronate sodium 70 mg are bioequivalent in terms of rate and extent of absorption.
CHRONIC INFLAMMATORY DISEASES: RHEUMATOID ARTHRITIS

P189
BI RECEPTOR AS A TARGET FOR DEVELOPMENT OF NOVEL ANTI-INFLAMMATORY AGENTS: EFFECT OF DBK AND ITS ANALOGUES ON MICE STOMACH FUND
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Introduction: Kinin B1 receptor (B1R), a seven transmembrane domain, G protein-coupled receptor is involved in inflammation and nociception in response to its agonist des-Arg9-bradykinin (DBK - Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe). Recent studies on the structure-activity of the B1R revealed that important residues for its binding to DBK are homologous to those in the interaction between angiotensin II (AngII) and its type I receptor (AT1R). As DBK and AngII have the same two residues at the C-terminal region (Pro7-Phe8) we hypothesized that the two peptides present a common crucial points of interaction with their receptors. Thus our study was to identify the role of important residues for the binding of DBK to B1R based on some residues playing critical roles in the interaction AngII-AT1R.

Methods: DBK analogues were synthesized replacing each amino acid of the molecule for alanine. The role of Arg1 substituted by Lys (Lys1DBK) and of Gly4 by Leu (Leu4-DBK) were also assessed. Biological activity was tested determining the concentration-contraction curves for DBK analogues using slices of mice stomach fundus that constitutively expresses B1R.

Results: It was found that the potency and the intrinsic activity induced by Ala1-DBK- and Leu4-DBK- induced effects were abolished but the effect of Lys1-DBK was higher than DBK. The activity of Ala6-DBK e Ala7-DBK was altered whereas a reduction was observed for Ala2-DBK, Ala3-DBK and Ala8-DBK. The potency but not the maximal effect was reduced for Ala4-DBK and Ala5-DBK.

Conclusions: These results indicated that the positions 2, 4 and 8 play important roles as receptors for AngII. It is concluded that the activity of B1R depends on epitope similar to that proposed for the AT1R. Our findings provide perspective in the development of new antagonists as effective anti-inflammatory agents acting through the B1R. Supported by FAPESP and CNPq.

P190
NEW POLY-HETEROCYCLIC COMPOUNDS WITH ANTI-INFLAMMATORY POTENTIAL EVALUATED IN AN ARTHRITIS MODEL INDUCED WITH FREUND ADJUVANT
Mogosan C.1, Vostinaru O.1, Parvu A. E.2, Zaharia V.3, Ignat A.3
1Department of Pharmacology, Physiology and Physiopathology, Faculty of Pharmacy, University of Medicine and Pharmacy Iuliu Hatieganu, Cluj-Napoca, Romania

Introduction: The anti-inflammatory activity of five new poly-heterocyclic compounds with a thiazolic ring (S1-S5) was determined in a chronic immune inflammation model.

Methods: The experimental model of arthritis induced with Freund adjuvant in the rat, was used. The tested compounds (S1–S5) have been administered intraperitoneally, 40 mg/kg for 7 days, from the 18th day after the induction of the arthritis. The volume of the inflammatory oedema was determined plethysmometrically in the paw treated with Freund adjuvant and also in the opposite one, and at the end of the experiment, blood samples were taken in order to determine oxidative stress parameters.

Results: The primary inflammatory response was significantly reduced in the rats treated with S3 and S4 compounds. The secondary inflammatory response was reduced in the rats treated with S1, S2 and also S5 compounds. Moreover, S1, S2 and S5 compounds have demonstrated antioxidant properties, but the S3 and S4 compounds increased the oxidative stress.

Conclusions: The studied compounds demonstrated anti-inflammatory effects in an arthritis model induced with Freund adjuvant, presenting themselves as potential therapeutic agents.

Acknowledgement: This work was supported by CNCSIS-UEFISCSU, project number PN II-IDEI code 1269/2008.

P191
SYNTHESIS AND STUDY THE ANALGESIC AND ANTI-INFLAMMATORY EFFECT OF RIGID 6 METHOXY BENZOPYRAN 3, 4 DIHYDROXY CHALCON (DHC) IN MICE
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Background and Aims: Although, anti-inflammatory and antinociceptive drugs have some useful applications, they have some adverse effects. Therefore researchers are trying to find new anti-inflammatory compounds with more ideal applications and less adverse effects. In this research anti-inflammatory and antinociceptive effects of rigid derivative of 6-methoxy benzopyran 3,4- Dihydroxy chalcone (DHC) was evaluated.

Methods: At first different doses of DHC 25, 50 and 75 mg/kg were injected to mice and the analgesic and anti-inflammation effects of it was evaluated by by Formalin, Hot plate and Carageenan tests.

Results: The result showed that dose of 50 mg/kg of DHC induced more significant anti-inflammation and antinociception in formalin test. In addition the effect of DHC was higher in the chronic phase, therefore it seems that DHC has better anti-inflammatory effect rather than analgesic effect. Doses of 50 and 75 induced some lethargy in the mice. DHC induced analgesia and anti-inflammation in hotplate and carageenan test too.

Conclusion: The result showed that DHC has a significant anti-inflammatory and antinociceptive effect and more effective compounds can be synthesized by modification of structure of this derivative. This investigation was supported financially by research center of kerman university f medical sciences.

Keywords: 3,4-dihydroxychalcon (DHC), Anti inflammation, Anti nociception, Mice.

P192
EFFICACY AND SAFETY OF EPERISONE HYDROCHLORIDE IN TREATMENT OF MUSCULOSKELETAL SPASM ASSOCIATED WITH CERVICAL SPONDYLOSIS: AN OP
1Eisai Pharmaceuticals India Private limited, Mumbai, MS, India; 2Department of Orthopedics, Grant Medical College & Sir J

Introduction: Eperisone, a b-aminopropriophenone derivative, is a centrally acting skeletal muscle relaxant useful in relieving myotonic symptoms like stiffness, cervical pain, muscle and low back pain. The present study was conducted with an aim to investigate efficacy & tolerability of Eperisone in treatment of patients with acute musculoskeletal spasm associated with cervical spondylosis.

Methods: This was a prospective, open-labeled, non-comparative, multicentric, observational study. Patient suffering from acute musculoskeletal spasm associated with cervical spondylosis, attending out-patient &
P193 EVALUATION OF SAFETY & EFFICACY OF EPERISONE-HCL IN TREATMENT OF ACUTE MUSCULOSKELETAL SPASM, ASSOCIATED WITH PERIARTHROSIS HUMEROSCAPULARIS
1Eisai Pharmaceuticals India Private limited, Mumbai, MS, India; 2Department of Orthopedics, Grant Medical College & Sir J

Introduction: Eperisone is a skeletal muscle relaxant acting on spinal cord with additional vasodilation property. It is useful in relieving myotoxic symptoms like stiffness, cervical pain, muscle contraction headache, and low back pain. The present study was conducted to evaluate the safety and efficacy of eperisone hydrochloride in the treatment of acute musculoskeletal spasm associated with periarthritis humeroscapularis.

Materials & Methods: In this open, prospective, non-comparative, post-marketing surveillance study, 108 patients with stresses shoulder receiving eperisone hydrochloride 150 mg/d for 4 weeks. Assessments were made for shoulder pain, functionality (activities of daily living), range of motion in sitting position (active flexion, extension, abduction, adduction & external rotation) and shoulder joint stability. Diclofenac & acetylsalicylic acid were the most common rescue medications used, if required. Safety was assessed by the adverse events reported. Global assessments were made at the end for the clinical response (CGART) by clinician and tolerability to eperisone (PGATT) by patient. Analysis of parameters was done by one way ANOVA (repeat measures) and Friedman test (non-parametric).

Results: Ninety patients completed the study of which 41 (45.6%) needed rescue for pain. Significant (P < 0.0001) improvements from baseline were seen in shoulder pain (87.9%), functionality (52.9%), range of motion for all movements and shoulder joint stability (17.5%). Global response, by clinicians was reported good to excellent in 84.2% patients & in 4 (5.3%) patients the response was poor. Adverse events were mild to moderate in intensity and were reported in 7.78% of study population, with most common being dry mouth, vomiting, dizziness and tininitus.

Conclusion: Eperisone is effective and well tolerated in patients with musculoskeletal related with periarthritis humeroscapularis.

P194 SPINAL AND SUPRASPINAL ANTINOCICEPTIVE AND ANALGESIC EFFECTS OF MILNACIPRAN IN FIBROMYALGIA
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Introduction: Fibromyalgia is linked to dysfunctions of nociception. Milnacipran, a SNRI, was shown to be effective in reducing pain in fibromyalgia and to increase activity in brain areas associated with descending inhibitory pain pathways.

Methods: Randomized, placebo-controlled double-blind study evaluating antinociceptive effects of milnacipran following 7-week exposure (100, 150, 200 mg/day) in female fibromyalgia patients. Evaluation included Nociceptive Flexion Reflex (NFR), Cold Pressor Test (CPT), and self-reported questionnaires such as Weekly-Recall Pain VAS (VAS), fibromyalgia impact (FIQ), health-related quality of life (SF-36 and PGWB), depression (BDI), anxiety (STAI), patient’s global impression of change (PGIC). Covariance analysis was used on primary and secondary criteria.

Results: Seventy-seven (39 Placebo, 38 Milnacipran all doses), out of 80 randomized patients, were available for analysis. On the NFR, there were no differences between the two groups. However, consistent with previous studies, the Milnacipran treated group reported a 16.5 mm reduction in pain score (VAS) vs. 4.1 mm in the placebo group (P < 0.05). The adjusted change difference in pain reduction (VAS) between placebo and the tertile with the highest plasma levels of Milnacipran was 34.2 mm (P < 0.05). BDI and STAI scores remained unchanged in Milnacipran. Self-reported questionnaires consistently reflected positive effects of Milnacipran on quality of life (SF-36) and psychological well-being (PGWB). Odds Ratio (OR) 5.1 for PGIC responders (i.e. very much improved, much improved) was greatly in favour of Milnacipran (P < 0.05).

Conclusion: Milnacipran has a predominantly supraspinal analgesic effect as evidenced by the absence of nociceptive spinal reflex changes. Higher plasma concentration was associated with higher pain reduction. Reported analgesia was independent of patients’ emotional status.

P195 IBUPROFEN AND COMPOUND ACTION POTENTIAL
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Introduction: Study is designed to examine effect of ibuprofen cream in solution form, on frog sciatic nerve compound action potential (CAP) parameters.

Materials: CAP parameters were measured using extracellular recording technique, stimulator and digital storage oscilloscope. Frog sciatic nerves were divided into three groups, eight nerves in each group. Nerves were incubated in Ringer solution for 2 h and control measure were done. Then, nerves were incubated for 20 min in following solutions: control group: Ringer solution, group I: 0.075% and group II: 0.25% ibuprofen solution. CAP parameters were measured again. Data were analysed using the statistical computer program GraphPad Prism 5.0.

Results: After 20 min incubation in 0.25% and 0.075% ibuprofen solution amplitude of CAP decreased for 60.13 ± 5.12% and 44.16 ± 4.80%, onset latency period of the CAP increased for 24.05 ± 3.35% and 13.27 ± 1.31% and peak latency time of the CAP for 37.24 ± 2.04% and 21.74 ± 1.34%, compared to control group, respectively. Data were statistically significant in relation to control group for P < 0.01. All nerves recovered their function after 2 h incubation period in Ringer solution.

Conclusion: Study results showed that ibuprofen solution statistically significant decreases the CAP amplitude and increases nerve conduction time, which confirms its effect on each nerve fiber. Ibuprofen may have an antinociceptive effect because it significantly prolongs the peak
Introduction: Eperisone is a skeletal muscle relaxant acting on spinal cord with additional vasodilation property. It is useful in relieving myo-
tonic symptoms like stiffshoulder, cervical pain, muscle contraction head-
ache, and low back pain. The aim of present study was to investigate
efficacy & tolerability of Eperisone hydrochloride, a β-aminopropiophene
derivative, in treatment of patients with acute musculoskeletal spasm associated with low back pain.

Methods: This was a prospective, open-labeled, non-comparative, mul-
ticentric trial. Patient attending out-patient settings of neurology, neuro-
surgery, internal medicine, traumatology and orthopedics were treated 
with eperisone 50 mg thrice daily for 4 weeks. Patients were evaluated 
for objective & subjective parameters, need for rescue medication and 
response to therapy for efficacy & tolerability.

Results: Of total 357 patients enrolled, 339 were available for intent-to-
treat analysis. There was significant improvement in all objective param-
eters of finger-to-floor distance, Lasègue's sign positive, hypermyotonia, tenderness, leg tendon reflexes and subjective parameters of lumbar 
cinelesia, pain in lower limbs, sensory symptoms like tingling, numb-
ness and paresthesia at end of study visit (P < 0.01 vs baseline). Total 
94.37% of patients responded therapy as good to excellent. The therapy 
with eperisone was well tolerated with all adverse events being mild to 
moderate in intensity. Most commonly reported adverse events in both 
groups were nausea, abdominal pain, headache & dizziness. At end-visit 
evaluation 35.71% in eperisone group required rescue medication as 
against 73.45% in placebo group (P < 0.01 vs placebo), demonstrating 
analgesic efficacy of eperisone.

Conclusion: The study demonstrates eperisone hydrochloride to be effective & well tolerated in treatment of patients with acute musculo-
skeletal spasm associated with low back pain.

Introduction: Eperisone is a skeletal muscle relaxant acting on spinal 
cord with additional vasodilatation property. It is useful in relieving myo-
tonic symptoms like stiffshoulder, cervical pain, muscle contraction head-
ache, and low back pain. The aim of present study was to investigate
P198
IMPACT OF THE USE OF DOCTOR-PATIENT COMMUNICATION VIDEO RECORD DURING RATIONAL PHARMACOTHERAPY TRAINING FOR FOURTH-YEAR MEDICAL STUDENTS
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Introduction: In academic year 2008/2009, University of Dokuz Eylül Medical School decided to introduce a new course on rational pharmacotherapy (RPT). A 5 day-RPT course was integrated to the curriculum in the beginning of the fourth-year.

Methods: The impact of the RPT course on prescribing skills was measured by pre/post-test design by an objective structured clinical examination (OSCE). Problem solving (80 points) and communication skills (20 points) were assessed with OSCE. In 2008/9 and 2009/10 academic years, mean scores of the post-tests were significantly higher than the pre-tests, and problem solving skills score increased from 14.4 ± 0.6 to 54.9 ± 0.8 (P < 0.0001, n = 267). In academic year 2010/11, to further improve problem solving skills score of the students we added doctor-patient consultation video record to the RPT course programme. During training the students were asked to evaluate the doctor-patient communication and prescription on two video records using a checklist followed by group discussions. One of the records demonstrated perfect and the other one inappropriate communication and prescription methods.

Results: Total post-test OSCE score was significantly higher for 2010/2011 academic year students (n = 155) than 2008/2009 and 2009/2010 year students (n = 267) (P < 0.0001). Problem solving skills score also improved from 54.9 ± 0.8 to 76.3 ± 1.0 (P < 0.0001).

Conclusions: The present study demonstrated that the fourth-year medical students markedly benefited from video record and group discussion in developing rational prescribing skills.

P199
LONGITUDINAL EVALUATION OF THE THERAPEUTIC COMPETENCE OF MEDICAL STUDENTS DURING CONTEXT-LEARNING UNDERGRADUATE MEDICAL TRAINING
VU University Medical Center Amsterdam, The Netherlands

The VU University Medical Center Amsterdam, The Netherlands, has developed a context-learning course in Clinical Pharmacology and Therapeutics (CPT) which is offered in years 2–6 of the undergraduate medical curriculum. Context learning is defined as learning in the setting of the future profession, i.e. clinical patient consultations. Throughout undergraduate training, the real-life nature of the setting is increased:
1 ‘Consultations’ with written case histories in years 2 and 3;
2 Role-play consultations and consultations with simulated patients in year 4;
3 Consultations with real patients during clinical clerkships and internships in years 5 and 6.

As part of the end-of-year examination, students carry out a consultation in the practice setting trained in that year. In order to evaluate the progress of each student, the level of therapeutic competence is evaluated annually, using a standard scoring form based on the WHO 6-step. In addition to students’ therapeutic competence, their knowledge of therapeutics is tested. Students also rate their own therapeutic competence and their appreciation of the teaching programme.

The CPT course will be presented in more detail at the congress. In addition a video fragment of a therapeutic consultation will be shown, carried out by a student during an examination. The results of the first evaluation from May 2010 to June 2011 will be presented and discussed.

P200
STRUCTURING THE THERAPEUTIC SECTION OF THE MEDICAL RECORD AS AN EDUCATIONAL TOOL
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Introduction: High rates of prescribing errors are an international problem. A major cause of prescribing errors is a lack of information in the medical record. Moreover, there appears to be discrepancy between the therapeutic information that clinicians think should be recorded and what they actually record. Several studies have shown that a medical record template improves the completeness of documentation. The goal of this study was to develop a pre-designed form for the therapeutic section of the medical record, mainly for educational reasons. To this end, we first investigated what therapeutic information doctors consider essential to include in the patient medical record.

Method: A two-round Internet Delphi study among 147 specialty registrars and 85 consultants in internal medicine from six Dutch teaching hospitals was used to achieve consensus. Fifty-nine items were assessed on a five-point scale; an item was considered important if ≥80% of the respondents awarded it a score of 4 or 5.

Results: In total 26 (18%) specialty registrars and 30 (35%) clinical consultants completed both rounds of the study. Seven items were considered important to include in the medical record by both clinical consultants and specialty registrars (see table). Specialty registrars also considered 7 additional items as important.

| Table 1. Important items that should be included in the medical record |
|---------------------------|-----------------------------|
| Indication for therapy   | Non-drug therapy            |
| Drug therapy             | Start and stop date         |
| Check for interactions and contra-indications | Patient has received information |
|                           | Monitoring appointments     |

Conclusion: The medical record should contain a structured therapeutic section that incorporates the seven items that were considered important by both clinical consultants and specialty registrars. An example of a structured medical record including these items will be presented.

P201
CLINICAL PHARMACOLOGY: AS A PART OF GRADUATE TEACHING AT THE UNIVERSITY OF MEDICINE AND PHARMACY MAROSVÁSÁRHELY
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The University of Medicine and Pharmacy Marosvásárhely has begun his activity in 1945. Since then pharmacological teaching is an integrated
part of medical development. Scientifically recognized professors have preceded the current staff at the pharmacology department. Regarding clinical pharmacology the history is not so significant. In Romania clinical pharmacology has become a possible choice for residency only in 2000, but clinical pharmacology courses have been held in Marosvasarhely since 1975. The challenge of teaching clinical pharmacology has belonged to different departments through time: at first to the pharmacology department, after that to the internal medicine department and finally now again to the pharmacology department. Although it is still an optionally taken subject, the aim is to introduce the students in the world of this science. In present there are >10 clinical pharmacologists and specialty residents in our university center, and the role of these specialists is not well known. Our purpose is to extend the knowledge and recognition of clinical pharmacology: therefore we are trying to realize complex courses and to set up research activities. Also there are no real PhD theses for the time being in this topic – we are hoping to make way shortly in this area. By presenting our history and objectives we are expecting to get more ideas to realize a proper teaching environment, but also we have set up a project for ourselves.

P202

EMTRAIN-EUROPEAN MEDICINES RESEARCH TRAINING NETWORK

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The Innovative Medicines Initiative (IMI) strategic research agenda (http://www.imi.europa.eu) identified gaps within education and training in support of the medicines development process. The objective of EMTRAIN is to establish a pan-European platform of excellence for education and training covering the whole life cycle of medicines from basic research through clinical development to pharmacovigilance. As a public-private-partnership the EMTRAIN consortium consists of six pan-European biomedical research infrastructures from the ESFRI roadmap and fifteen EFPIA (http://www.efpia.org) companies. Based on extensive mapping of existing resources and the results of subsequent gap analyses, the group has developed a strategy for the harmonization and accreditation of Master level and PhD programmes as well as for continuous education programmes, and is implementing a comprehensive course catalogue. In collaboration with a variety of stakeholders, EMTRAIN will ensure that training programmes are tailored in a coordinated way for current and future professionals involved in biomedical research and development. Flexibility and mobility of scientists is required to take advantage of the varied opportunities at the pan-European level and to understand European needs in pharmaceutical medicine. This capacity-building project will build up the infrastructure required and will facilitate mobility between academia, industry and regulators. National implementation will be facilitated through contacts with university authorities, ministries of higher education and through national liaison offices. Pan-European extension of the Network is planned. The harmonization and modular nature of these programmes will allow trans-disciplinary curricula as well as trans-border mobility, and PhD programmes are being designed to foster greater industry/academia understanding, mobility and collaboration. This total package is designed to boost Europe’s competitiveness in medicines research and to re-establish its position as a global leader in this field. (www.emtrain.eu)

P203

IMI PHARMATRAIN PROJECT – MASTERING MEDICINES DEVELOPMENT

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Semmelweis University Budapest on behalf of the IMI Pharmatrain Cooperation, Member of the Executive Board of PharmaTrain

PharmaTrain (Pharmaceutical Medicines Training Programme) is the new European platform for postgraduate training in Pharmaceutical Medicine/Drug Development Sciences and fosters the training needs for all professionals in this field. The main objective of PharmaTrain is to harmonize newly created as well as existing Base Course and Master Programmes based on the Bologna credit system (30, 60 and 90 ECTS programmes). Courses will improve the knowledge by providing the helicopter view on drug development on the one hand and in-depth knowledge in all aspects of the integrated drug development process on the other. The PharmaTrain project has started 2009 with 22 European universities, 14 learned societies including three regulatory authorities and 15 EFPIA partners. Partners in PharmaTrain including the Course providers in a first step have agreed on a PharmaTrain Syllabus followed now by curriculum, learning outcomes and quality standards as well as an e-campus for blended learning. After 2 years into the PharmaTrain project, 15 universities providing both Base Courses and Master Programmes are now in the process to implement the shared standards and best practices, one major milestone in the programme. A new postgraduate Masters degree was born in Europe – the Master in Medicines Development, MMD, which will become known more and more. Additional focus lies on the geographic extension of the Master programme in new European Countries by creating a Cooperative European Drug Development Course (CEDDC). The PharmaTrain e-Campus with an e-library and the CPD platform has been created for both trainees and trainers and is available on www.pharmatrain.eu.

P204

IMI SAFESCIMET PROGRESS AND CHALLENGES EUROPEAN MODULAR EDUCATION AND TRAINING PROGRAMME IN SAFETY SCIENCES FOR MEDICINES

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Safety issues are major reasons for discontinuing otherwise promising drug candidates from pharmaceutical development of new medicines. The ability to better forecast unacceptable adverse events and thus avoiding problems in later phases and unnecessary development costs requires informed use of technology, developed in different areas, and their adaptation to predicting across species properties relevant for safe use of a therapeutic principle. Today’s education and training of safety scientists and specialists need a stronger focus on the development and application of such new methodologies. In November 2010, the IMI SafeSciMET (www.safescimet.eu) started delivering the first pan-European education and training programme course module in safety sciences, on Drug Discovery and Development. In the first half of 2011, five more modules will follow on Pharmaceutics and Safety; on Pharmacokinetics and Dynamics; on Laboratory Animals, Alternatives and Ethics; on Biochemical and Molecular Toxicology; Biotransformation, Bioactivation and Adverse Drug Reactions; and on Cellular Toxicology/Predictive Toxicology; respectively. In all it is made up of 20 course modules to be run over the next 2 years (2011–2012), enabling formation of specialists with the capabilities to perform a holistic and critical evaluation of the safety of drug candidates and new medicines. It is integrating animal and human data and relevance. This unique partnership between European universities and leading pharmaceutical companies ensures that the available knowledge in academia and in industry be combined with modern competence-directed learning approaches. Real-life case studies complement class-room lectures in all major preclinical and clinical safety areas. The modular structure of the teaching programme allows for flexible selection of courses from industry, academia and regulatory bodies to select and attend course modules identified as essential for individual continuous professional development (CPD), or to attend the full programme leading up to an Advanced MSc degree in Safety Sciences. Each SafeSciMET course module is built up of 1 week of on-site teaching and 1 week of self-training and coached distance learning, taking advantage of industry-supplied case studies and using a variety of teaching modalities, plus a written examination (for the MSc). The SafeSciMET consortium comprises 18 public partners and 15 industry partners. (www.safescimet.eu).
P205
EU2P – EUROPEAN PROGRAMME IN PHARMACOVIGILANCE AND PHARMACOEPIDEMIOLOGY
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EU2P aims to improve the understanding of medicines-related risk by developing a European training and education platform in Pharmacovigilance and Pharmacoeconomics for academia, industry and regulatory bodies. This challenge was embraced by seven Universities, the European and French Medicines Agencies and fifteen Pharmaceutical Companies who have put their strengths together in order to build the EU2P consortium. This programme offers courses in Pharmacovigilance and Pharmacoeconomics with specialties in benefit assessment, regulatory aspects, risk quantification, public health and risk communication in order to deliver certificates recognised by EU2P academia, industry and regulatory bodies and new postgraduate diplomas in the framework of the Bologna process. Initiated in Sept. 2009, EU2P is building up its education programme to offer first course deliveries in Autumn 2011. EU2P targets specialists such as pharmacists, physicians, scientists and experienced professionals but also non specialists such as media-members, laypersons and patients especially for risk communication training. EU2P users build custom training programmes that can lead to certificates, a full master’s or a PhD according to the options chosen. Emphasis will be put on hands-on training to maximise post-training employment opportunities. EU2P courses will be delivered in English using a unique and innovative modular approach integrating face-to-face lectures, e-teaching, and e-learning formats through the Eu2P e-learning platform. By 2014, EU2P training programme should be self-sustaining through excellence. Its consortium should host and include new European and international partners (http://www.eu2p.org).

P206
ZAGREB MEDICAL STUDENTS’ ATTITUDES TOWARDS ONLINE FORMATIVE ASSESSMENT QUIZZES IN A COURSE ON RATIONAL MEDICAL THERAPEUTICS
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In the academic year 2009/2010, an electronic problem-based therapeutic course developed at the University of Michigan Medical School (UMMS) was in a collaborative manner translated and adapted for use of the 250 final year students at the University of Zagreb Medical School (UZMS). In all, it comprised 90 clinical cases distributed over 20 thematic modules and 300 distinctive FLASH video commentaries totaling around 6 h of video and around 1000 web pages (clinical cases, MCQ answer feedback, further resources), all distributed to students through the ctools learning management system (LMS). In order to successfully finish the course students had to pass 22 weekly online quizzes spread over 4 weeks (on average five quizzes per week), which were offered in a timed manner (20 min were allowed per quiz question) using Questionmark’s Perception software package. Students took them in an open book format and could access them within the week they were offered when ever it was convenient to them. At the beginning of the course all students attended a 90 min long tutorial where they were shown how to access and browse the tools as well as take a quiz through Questionmark’s platform. Students’ Perception login details were kept distinctively different from their ctools user details, hence every student received a separate username and password for access to the online quiz system. A designated team of administrators and instructors dealt with students’ access and quiz problems on an individual basis. While course quality assessment demonstrated high satisfaction of Zagreb students with the provided teaching format (4.11 ± 0.86; 1 = poor to 5 = excellent) it also revealed that they considered weekly online quizzes to be rather difficult (3.83 ± 0.82; 1 = Too Easy to 5 = Too Difficult) but nevertheless found it very useful to receive immediate feedback regarding their submitted quizzes (4.17 ± 0.85; 1 = Strongly Disagree to 5 = Strongly Agree). From the instructors perspective, with only 3% of the total amount of students’ quiz attempts being reported back for administrators’ review and support, we believe online formative assessments have great potential to cost effectively contribute to a significant, individual, and safe gain in learners’ therapeutic skills prescribing skills in an environment that can be designed to be very close to real life medical practice.

P207
TOWARDS BETTER UNDERGRADUATE MEDICAL EDUCATION: ARE MEDICAL STUDENTS AWARE OF DRUG SAFETY RISK?
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Department of Pharmacology, Clinical Pharmacology and Toxicology, School of Medicine, University of Belgrade, Dr Subotica staraj
We aimed to assess the attitudes of undergraduate medical students regarding drug safety risk. In this cross-section study, two samples of students were questioned on adverse drug reactions (ADRs): a self-completed questionnaire was delivered to 69 out of 511 students of the 3rd year (13.50%), and 98 out of 508 students of the 6th year (19.29%). Total response rate was 99.19%. As expected, 6th year students considered to be better informed on ADRs than the 3rd year students (P < 0.0001). The 6th year group considered the pharmacists as a significantly less reliable source of information on ADRs, and the patients as a more reliable source than the 3rd year group (P = 0.0043, and 0.0148, respectively). Both groups similarly assessed the risk from the use of different drug classes, considering anxiolytics/hypnotics as the highest risk drugs, and aspirin as the lowest risk one (visual analogue scale, VAS 1–10; medians of 9 and 4 in 3rd year group, and 8 and 5 in 6th year group). However, 3rd year students considered the risk of the use of anti-hypertensives, hormone contraceptives, and anxiolytics/hypnotics significantly higher than 6th year students. Finally, 6th year students were more confident regarding the self-medication with antibiotics (P = 0.0189), but assessed that the risk of self-medication was very high (VAS 1–10; median of 9). In conclusion, undergraduate pharmacology courses significantly influenced the opinion of medical students on the risk of ADRs. However, significant improvement is still needed in our undergraduate medical education regarding drug safety awareness.

P208
TEACHING GOOD CLINICAL PRACTICE IN SLOVAKIA: MULTIDISCIPLINARY, STATE-ACREDITED POSTGRADUATE EDUCATION PROGRAM
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Teaching both Good Clinical Practice (GCP) and research ethics in multidisciplinary settings has more than 15-years long tradition in Slovakia. Since the very beginning of the establishment and work of research ethics committees (ECs) (since 1990/1991), their members were invited, together with other acting parties involved in biomedical research projects (including drug clinical trials – CTs) to common multidisciplinary education courses and conferences. Among the participants and/or faculty of those activities, the following professional categories were regularly enlisted: investigators, researchers, monitors, medical or research departments’ employees of pharmaceutical companies, representatives of the Slovak competent authority (State Institute of Drug Control – SIDC) and of the Slovak Ministry of Health. The aim of these activities was to educate and train representatives of all important ‘players’ in the field of
GCP and ethics review together, thus to establish in a ‘natural way’ the good communication and exchange of information, while simultaneously building common understanding and commitment in developing and sustaining good practices in CTs’ realm, as well as to enable transparency and good quality in ethics review of biomedical research projects and CTs’ protocols. Building on the accumulated experience and reflecting the growing needs in the area, a state-accredited certification program has been developed and hosted by the Institute of Pharmacology, Clinical and Experimental Pharmacology of the Slovak Medical University in Bratislava (the official state accreditation awarded by the Ministry of Health in 2003) in collaboration with the Institute of Medical Ethics and Bioethics. The program education and training activities should meet defined theoretical standards and practical skills requirements. The program is completed by the state board examination and successful participants are awarded the State Certificate in ‘Certified Working Method – Drug Clinical Investigation’. The certificate, issued by the Slovak Medical University, is becoming required to be submitted to SIDC by principal investigators, when SIDC is authorizing the pre-selected trial sites in Slovakia. It is also expected to become a necessary requirement for at least the chairs of the state registered (or accredited) research ethics committees under the pending ministerial regulation.

P209
TEACHING GOOD CLINICAL PRACTICE IN SLOVAKIA: MULTIDISCIPLINARY, STATE-ACCREDITED POSTGRADUATE EDUCATION PROGRAM

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Teaching both Good Clinical Practice (GCP) and research ethics in multidisciplinary settings has more than 15-years long tradition in Slovakia. Since the very beginning of the establishment and work of research ethics committees (ECs) (since 1990/1), their members were invited, together with other acting parties involved in biomedical research projects (including drug clinical trials – CTs) to common multidisciplinary education conferences and courses. Among the participants and/or faculty of those activities, the following professional categories were regularly enlisted: investigators, researchers, monitors, medical or research departments’ employees of pharmaceutical companies, representatives of the Slovak competent authority (State Institute of Drug Control – SIDC) and of the Slovak Ministry of Health. The aim of these activities was to educate and train representatives of all important ‘players’ in the field of GCP and ethics review together, thus to establish in a ‘natural way’ the good communication and exchange of information, while simultaneously building common understanding and commitment in developing and sustaining good practices in CTs’ realm, as well as to enable transparency and good quality in ethics review of biomedical research projects and CTs’ protocols. Building on the accumulated experience and reflecting the growing needs in the area, a state-accredited certification program has been developed and hosted by the Institute of Pharmacology, Clinical and Experimental Pharmacology of the Slovak Medical University in Bratislava (the official state accreditation awarded by the Ministry of Health in 2003) in collaboration with the Institute of Medical Ethics and Bioethics. The program education and training activities should meet defined theoretical standards and practical skills requirements. The program is completed by the state board examination and successful participants are awarded the State Certificate in ‘Certified Working Method – Drug Clinical Investigation’. The certificate, issued by the Slovak Medical University, is becoming required to be submitted to SIDC by principal investigators, when SIDC is authorizing the pre-selected trial sites in Slovakia. It is also expected to become a necessary requirement for at least the chairs of the state registered (or accredited) research ethics committees under the pending ministerial regulation.

P210
KNOWLEDGE AND ATTITUDES OF PHARMACY AND MEDICAL STUDENTS TOWARD COMPLEMENTARY AND ALTERNATIVE MEDICINE

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Introduction: Several previous studies have discussed the importance of determining the attitudes and perceptions of students and/or health care professionals toward complementary and alternative medicine (CAM). In view of the recognition that CAM in its various forms is enjoying a growing popularity among the public in the Gulf region, there is a lack of information on the attitudes and perceptions of students and/or health care professionals toward CAM. This study was designed to determine the use of CAM modalities among pharmacy and medical students, their knowledge, perceived effectiveness and harmfulness about CAM modalities, their general attitude toward CAM, and the perceived barriers to CAM use and the need for CAM education.

Methods: A descriptive and cross-sectional study was conducted in faculties of pharmacy and medicine at Kuwait University, Kuwait. The sample size was determined using Java Applets for Power and Sample Size. A total of 250 randomly selected pharmacy and medical students (125 from each faculty) were approached to be included in this study. The response rate was 88.4%.

Results: CAM usage was reported by 55.2% of students, and mostly associated with females (OR: 4.4; 95% CI: 1.7–11.3). Herbal products were the most commonly used (37.6%; 95% CI: 31.2–44.3%). Knowledge about 11 CAM modalities was generally poor, even for those who respondents claimed to know most about. The knowledge about herbal products was significantly better among pharmacy students (OR: 2.0; 95% CI: 1.1–3.6). Massage, herbal products and prayer/Qur’an reciting were perceived as being the most effective, while cauterization as the most harmful. Attitudes toward CAM were positive; with about 80.0% believing that CAM includes ideas and methods from which conventional medicine could benefit. Lack of trained professionals and lack of scientific evidence were the most perceived barriers for CAM implementation. About 90% admitted the importance of knowledge about CAM for them as future practitioners.

Conclusions: The use of various CAM therapies was reported by the students, and the general attitude toward CAM was positive. Their knowledge, perceived effectiveness and harmfulness about CAM modalities were diverse. The students acknowledged the need to be well educated about CAM to better advise their patients in the future.
**P211**

A HOSPITAL-WIDE PROGRAM TO OPTIMIZE ANTIBIOTIC USE IN RESPONSES TO AN INCREASING RATE OF CEFUROXIME RESISTANT KLEBSIELLA PNEUMONIAE

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**Background:** Certain bacteria produce Extended Spectrum Beta-Lactamase (ESBL), Klebsiella pneumoniae being one of the most important. ESBL makes bacteria resistant to commonly used antibiotics.

**Methods:** The intervention took place from January 2010 at a 600-bedded university hospital, >40% of K. pneumoniae isolates were ESBL producing. In collaboration with the microbiologist, the Drug and Therapeutic Committee developed a programme to restrict use of cephalosporines to surgical prophylaxis and meningitis. The programme comprised revision of antibiotic guidelines, new isolation precautions, information for patients and personnel, diagnostic procedures, and training sessions. Project activities were monitored and corrective actions taken, when necessary.

**Results:** From 2009 to 2010, mean cefuroxime and ciprofloxacin use decreased: 191 to 46 (P < 0.001) and 119 to 99 Defined Daily Doses per 1000 occupied bed-days (DDD/1000 OBD) (P = 0.13). Piperacillin-tazobactam and ertapenem use increased: 8 to 99 (P < 0.0001) and 17 DDD/1000 OBD (P = 0.0001).

At the control setting (405-bedded university hospital with a similar ESBL rate), cefuroxime and ciprofloxacin use remained constant. Piperacillin-tazobactam and ertapenem use increased slightly: 3.5–15 (P < 0.0001) and 2.0–4.0 DDD/1000 OBD (P = 0.030). Total antimicrobial use was constant at both settings.

The mean infection rate for ESBL producing K. pneumoniae decreased at the intervention hospital [(1.05–0.75 patients/1000 OBD (P = 0.0016)] and remained unchanged at the control hospital (1.11–1.06 patients/1000 OBD, P = 0.73).

**Conclusion:** Data suggest a beneficial impact of the Drug and Therapeutic Committee’s programme on antibiotic use and hospital infection rate with ESBL producing K. pneumoniae.

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**P212**

PRESCRIPTION OF ANTI-INFECTIVE DRUGS IN PATIENTS ON CHRONIC IMMUNOSUPPRESSION THERAPY

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**Introduction:** Patients on immunosuppressive (IS) therapy have a higher risk of infection and are often treated with drugs presenting possible drug-drug interactions (DDI) at the level of CYP450 metabolism. Interactions with anti-infective drugs (AID) pose higher risk as their prescription is episodical and level fluctuations are therefore more likely. We set out to investigate the extent to which such interactions are common and clinically relevant.

**Method:** We analyzed close to 2 million prescriptions issued in the University Hospital in Olomouc between Jan 2005 and December 2010 and identified patients on chronic IS therapy (defined as recurring prescription of at least 3 month duration). Anti-infective drug consumption was assessed in the period covered by IS prescription (defined as last prescription + 3 months). A total of 1185 IS patients were identified, average age 50 years, 50% male. Drugs were classified by their effect on CYP450.

**Results:** Although IS patients represent only around 2% of the hospital patient population, they amount to 10% of prescribed DDD of AIDs and close to 30% of the total AID costs. The structure of AID prescription is significantly different, with cotrimoxazole playing a larger role (prophylaxis) and a shift towards bactericidal drugs. Whereas potentially interact-

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**P213**

MEDICAL ERRORS IN PRESCRIPTION OF ANTIBACTERIALS IN CRIMEA, UKRAINE

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**Pharmacotherapy:** Antibacterial drugs continue to be most frequently prescribed pharmacological group in Crimea and they caused adverse reactions more often than any other class of medicines. We analyzed reports about side effects of drugs sent by doctors to us in last 3 years. We found that in 12% of sent reports informing about side effects of antibacterials they were prescribed with errors. The most frequent errors according third generation of cephalosporins are unjustified use of them for prevention of infection during surgical operations, administration of them in patients with infections caused by gram-positive bacteria non-included in spectrum, administration of them in patients with viral respiratory infection and incorrect regimen of therapy when drugs were prescribed two times per day and even two times per day instead of once a day. In 60% of patients these errors led to severe complication and patients were hospitalized. In 15 of 18 cases when cefalexin was prescribed when gram-negative infection took place and pharmacotherapy was ineffective. In fluoroquinolones group it was found that first generation drugs were used in patients with soft tissue infection and community-acquired pneumonia, for intraoperative prophylaxis (one case) and in children (four reports), four anaphylactic shocks were caused. For penicillins and aminoglycosides errors were: ignoring of allergy anamnesis, combination of two penicillins (ampicillin sulbactam and amoxicillin clavulavonate) use of aminoglycosides for intraoperative prophylaxis and combination of two nephrotoxic drugs. Analysis of clinical situation shown that in all cases doctors had alternative less toxic drugs.

**Conclusion:** Medical errors are still one of the most frequent cause of hospitalization or prolongation of it, they also may increase adverse reaction risk.

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**P214**

PHARMACOKINETICS OF AMPICILLIN/SULBACTAM IN ELDERLY PATIENTS WITH COMMUNITY-ACQUIRED PNEUMONIA

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**Introduction:** Age-related physiological changes affect body systems, altering pharmacokinetics which may potentiate or alter the effects of
drugs. The aim of the study was to assess the influence of age on the steady-state pharmacokinetics of ampicillin/sulbactam in the population of elderly patients (≥65 years) with community-acquired pneumonia (CAP).

Methods: The pharmacokinetics of ampicillin/sulbactam were determined at steady-state in a total of 13 elderly (mean age 77.4 years, range 66–90 years) hospitalized patients with CAP following administration of multiple intravenous doses of 2 g ampicillin + 1 g sulbactam (Unacid® Pfizer) each over 15 min thrice a day. Blood samples were taken at baseline and serially over 8 h following infusion. Ampicillin and sulbactam concentrations were determined in plasma by HPLC-UV.

Results: The mean Cmax, AU(0-8h), total clearance, steady-state distribution volume, and half-life were 33.8 mg/l, 51.4 mg/h/l, 602.3 ml/min, 1.2 l/kg, and 2.1 h for ampicillin and 42.2 mg/l, 64.2 mg/h/l, 235.5 ml/min, 0.5 l/kg, and 3.3 h, respectively, for sulbactam. Reduced Cmax, AU(0-8h) and total clearance, prolonged half-life, and increased steady-state volume of distribution were observed for ampicillin. The mean free Cmin of 1.8 mg/l (95% CI: 0.6; 3.1) for ampicillin and 1.6 mg/l (95% CI: 0.9; 2.2) for sulbactam were greater than those predicted to be effective against the key pathogen of CAP Streptococcus pneumoniae (MIC90 1 mg/l). These results are consistent with a prolongation of antimicrobial activity of ampicillin-sulbactam in the elderly with T>MIC90 at a mean 92.3% (95% CI: 85.1; 99.6). For ampicillin the free Cmin/MIC90 ratio correlated positively with t1/2 of ampicillin and thus negatively with the creatinine clearance in elderly patients with CAP.

Conclusions: Age and conjectural pneumonia did affect the PK of ampicillin and sulbactam. Despite of reduced Cmax, adequate free Cmin/MIC90 ratios due to impaired renal function were observed in elderly patients with CAP. In elderly patients without renal impairment and/or in severe infection with less susceptible pathogens, more frequent dosing of ampicillin 2 g/sulbactam 1 g can be necessary to avoid the risk of under-dosing in CAP.

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ANTIMICROBIAL SUSCEPTIBILITY OF UROPATHOGENS IN OUTPATIENTS IN NORTHERN SERBIA

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Introduction: The aim of this study was to determine the distribution of uropathogens from outpatients living in North Serbia and the in vitro susceptibility of these organisms to antimicrobial agents.

Materials and Methods: The study was performed in Novi Sad, North Serbia (estimated population 350 000), over a 3-month period, from 1 January to 31 Mar 2008. We analysed the results of the susceptibility of 3282 uropathogen isolates.

Results: E. coli was the most frequently isolated pathogen, it was isolated from 65.60% urine samples, followed by Proteus mirabilis (8.74%) and Klebsiella pneumoniae (6.46%). The E. coli showed the highest sensitivity to cefixim (86.87%), ofloxacin (83.85%), norfloxacin (83.33%), cephalexin (82.96%), gentamicin (81.67%), and ciprofloxacin (80.72%), with low sensitivity to sulphametoxazole-trimethoprim (64.32%) and ampicillin (51.91%). The other bacteria presented lower sensitivity profiles. Conclusions: In conclusion, Escherichia coli was the most commonly isolated bacteria, and it was highly sensitive to cephalosporins, aminoglycosides and quinolones. Global trends of increase and dissemination of resistant strains of uropathogens show the necessity of keeping up the monitoring of antibiotic resistance in this part of Serbia.

Acknowledgement: This research is part of the project No. 41012 which is financially supported by the Ministry of Science of the Republic of Serbia.

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MULTI-LEVEL ANALYSIS OF RISK FACTORS FOR ESBL INFECTION IN THREE ICUS OF A TERTIARY HOSPITAL IN CROATIA

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Introduction: There is a dramatic increase in infections caused by extended spectrum beta lactamases (ESBL) producing strains of Escherichia coli (EC) and Klebsiella pneumoniae (KP), especially in intensive care units (ICUs). The aim of this comprehensive study was to adequately identify local risk factors for ESBL infection.

Patients and Methods: The study was conducted from June-2006 to July-2007 in 3 ICUs at three levels: (i) case-control study including all patients with KP/EC ESBL infection (cases) and patients with non-ESBL KP/EC infection (controls); (ii) ecological study of the impact of overall antibiotic consumption on the frequency of isolation of ESBL strains, and (iii) genotypization of a representative sample of isolates.

Results: Ecological study demonstrated that the use of fluoroquinolones and 3rd generation cephalosporins was significantly associated with the increase in frequency of ESBL strains isolation (time lag: 2 months), as well as the use of carbapenems, piperacillin/tazobactam, cephalosporins, aminoglycosides, glycopeptides and clindamycin (no time lag). Risk factors for ESBL infection identified in the case-control study were: duration of ICU stay, previous and the duration of previous antimicrobial therapy, number of prescribed antibiotics, previous use of macrolides and fluoroquinolones. Although there was no significant difference in mortality between cases and controls, patients who died in the case group more frequently received inadequate antimicrobial therapy. According to genotypization results, both selection pressure and horizontal transfer have been identified as important factors for acquisition of ESBL infection.

Results: Rationalization of antimicrobial consumption is urgently needed in the control of ESBL infections in ICUs.

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CEFIXIME VS. CIPROFLOXACIN FOR SHORT-TERM THERAPY OF ACUTE UNCOMPPLICATED LOWER URINARY TRACT INFECTIONS IN WOMEN

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Introduction: Uncomplicated urinary tract infections (uUTIs) are common in adult women across the entire age spectrum, with mean annual incidences of approximately 15% and 10%. A large range of antimicrobials in different doses are used in the treatment of uUTI.

Methods: A multicenter prospective randomized controlled trial was performed in two centres in Russia. Female patients (>18 years of age) with typical symptoms of acute uncomplicated lower UTI, without known structural or functional abnormalities of the urinary tract and without signs and symptoms of upper UTI and with bacteriuria (>103 CFU/ml) were recruited after giving informed oral witnessed consent. Patients were randomized to therapy with either cefixime (Suprax® Gedeon Richter, Hungary) 400 mg once a day for 5 days or ciprofloxacin (Ciproflo® Dr. Reddy’s, India) 250–500 mg twice a day for 5 days.

Results: A total of 104 patients (49 receiving cefixime and 55 receiving ciprofloxacin) with a mean age 33.8 ± 10.7 and 32.8 ± 12.1 years entered the study. During study was conducted transitional statistical analysis. Three to 4 days after therapy bacteriuria had been eliminated in 24/24 (100%) and 10/18 (55.6%) patients received cefixime and ciprofloxacin, respectively (P = 0.0003). After statistical analysis dose of ciprofloxacin was increased to 500 mg twice a day. Clinical cure and improvement were registered in 37 of 49 (75.5%) and 31 of 53 (58.1%) of the evaluable patients treated with cefixime and ciprofloxacin, respectively (P = 0.96). Bacteriological cure three to 4 days after therapy were registered in 47/49 (95.9%) and 35/53 (66%) patients.
received cefixime and ciprofloxacin, respectively (P = 0.0002). Both antimicrobial agents were generally well tolerated. Two patients (4.1%) in the group treated with cefixime (2 bacterial vaginosis) and eleven (20%) in the group treated with ciprofloxacin (7 - diarrhea, 2 - bacterial vaginosis, 1 - urticaria and 1 - pyelonephritis) experienced adverse events (P = 0.02).

Conclusion: Cefixime is a more effective and safe than ciprofloxacin in the treatment of acute uncomplicated cystitis in women.

P218
ACETYL SALICYLIC ACID STABILIZES IMMUNE FUNCTIONS OF MACROPHAGES
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Introduction: The impact of acetylsalicylic acid (ASA) as well as salicylic acid (SA) on important functions of macrophages was shown. For this approach the cell line RAW.264/7 was used. This cell line is highly standardized and shows many characteristics of primary macrophages and is therefore used in many laboratories to study characteristics of these cells. The data generated using RAW.264/7 are highly reliable and usage of primary monocytes or macrophages was not essential.

Methods: The highest ASA concentrations of the three tested concentrations (3.0, 0.003, 0.0003 mM) were predominantly most effective. For all experiments the xCelligence technology with RAW.264/7 cells was used.

Results: The results show an effect of ASA on the function of macrophages, a key population of the innate immunity. Other studies demonstrated an immune suppressive effect of even higher doses of ASA. However, the current study provides evidence for stimulation effects on macrophage mediated innate immunity. The highest dose that was tested demonstrates an immune suppressive effect of even higher doses of ASA. Especially the increased numbers of CD80/CD86 positive macrophages at eleventh treatment day.

Conclusion: UK

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ACYCLOVIR AND VALCICLOVIR EFFICACY FOR TREATMENT OF KERATOVAEITIS CAUSED BY HERPES SIMPLEX VIRUS, TYPE II
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Objectives: To evaluate the efficacy of acyclovir and valciclovir in adult patients with recurrent keratouveitis caused by herpes simplex virus, type II (HSVII).

Methods: A total of 70 adult patients (46 female and 24 male) 18–60 aged with recurrent keratouveitis caused by HSVII were included into the study during 2002–2006. All of investigated patients had 2–4 herpes simplex recurrences per year in medical history.

Antigen of HSVII was detected by immune fluorescent antibodies method in cell culture prior therapy, at seventh day and at eleventh day of treatment. Forty five patients were received valciclovir 500 mg twice daily orally for 14 days and twenty five patients were given 200 mg five times per day orally for 14 days.

Results: A good response as manifested by relief clinical symptoms (pain, redness, photophobia, permanent loss of vision, corneal oedema and keratic precipitates) and the beginning of their resolution was observed at fourth day in valciclovir-treated patients. Clinical improvement in patients treated by acyclovir occurred at sixth or seventh day. The patients of both treatment arms were positive for HSVII antigen at seventh day of therapy. Clinical recovery was determined at tenth day and at twelfth day in valciclovir-treated patients and acyclovir treated patients respectively. All of investigated patients were negative for HSVII antigen at eleventh treatment day.

Conclusion: The usage of valciclovir in patients with recurrent HSVII keratouveitis is more advisable due to more rapid clinical response and more stable remission.

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SWITCH FROM INTRAVENOUS TO ENTERAL MOXIFLOXACIN (400 MG) IN CRITICALLY ILL PATIENTS: A PILOT STUDY
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Introduction: In intensive care units, moxifloxacin is mainly used for the treatment of community acquired pneumonia. To achieve rapid bacterial killing, it is generally administered intravenously (IV). However, an early switch from IV to enteral or oral administration is favored not only because of pharmacoeconomic reasons, but also because of the benefits for the patient (i.e. reducing catheter-related infections). Although moxifloxacin demonstrated a good oral bioavailability in healthy volunteers, critically ill patients frequently exhibit physiological alterations which can affect the pharmacokinetic processes and, consequently, the efficacy of drugs. This study aims to investigate whether enteral administration of moxifloxacin is bioequivalent to IV administration in critically ill patients.

Patients and Methods: Four adult, critically ill patients receiving 400 mg of intravenous moxifloxacin once daily and being eligible for a switch to enteral moxifloxacin were enrolled in this single-centre, open-label, prospective bioequivalence study. Blood samples were obtained before and at serial time points after IV and enteral administration. The moxifloxacin plasma levels were measured by HPLC with fluorescence detection. Additionally, creatinine clearance was measured on each blood sampling day.

Results: In all patients, Cmax and AUC24h, and consequently Cmax/AUC24h, were lower after enteral administration compared to IV administration. Two patients (4.1%) had a noteworthy lower AUC24h/lg/ml than after IV administration (Cmax 10.7 ± 4.07 g/ml, AUC24h 40.13 ± 15.88 h*lg/ml) than after IV administration (Cmax 10.7 ± 4.07 g/ml, AUC24h 40.13 ± 15.88 h*lg/ml). Notwithstanding its preliminary character, this study demonstrated that, in critically ill patients, enteral administration of moxifloxacin is bioequivalent to IV administration in critically ill patients.

Conclusion: Notwithstanding its preliminary character, this study demonstrated that, in critically ill patients, enteral administration of moxifloxacin is bioequivalent to IV administration. Therefore, a switch from IV to enteral moxifloxacin administration does not seem to be recommendable in this patient population. Further studies are required to optimize moxifloxacin dosing in ICU patients.
P221
STEAM AEROSOLIZATION IMPROVED VASOCONSTRICTION RESPONSE IN LIPOPOLYSACCHARIDE-INDUCED SEPTIC RATS

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Introduction: Sepsis is a systemic response to an infection, characterised by peripheral arterial vasodilatation which leads to a hyperdynamic state with low systemic vascular resistance, high cardiac output, hypotension, and inadequate tissue perfusion. Hypotension during septic shock, relating to overproduction of nitric oxide, is often refractory to vasocostritcators. This study aimed to examine the effect of steam aerosolization on vascular response to vasoconstrictor in lipopolysaccharide (LPS)-induced sepsis in rat.

Methods: Male Sprague-Dawley rats were divided into four groups; control group, steam group (rat receiving steam aerosolization at 40°C, 1 h), LPS group (LPS 10 mg/kg i.p.) and steam-LPS group (rat receiving steam aerosolization at 40°C, 1 h before injection of LPS 10 mg/kg i.p.). Six hours after injection of LPS, rats were sacrificed and the aortas were immediately isolated for determination of vascular response to noradrenaline.

Results: The sensitivity and maximum response to noradrenaline of aortas isolated from the rats in LPS group were significantly decreased when compared to the control group. Aortas from rats receiving steam aerosolization at 40°C for 1 h before injection of LPS showed more sensitivity and response to noradrenaline compared with those in LPS group.

Conclusion: Our results suggested that impaired response to vasoconstrictr in LPS-induced sepsis in rats could be improved by inhalation of steam aerosolization, which may be effective therapeutic approach to the sepsis.

P222
MOLECULAR MECHANISM OF THE RENAL TUBULAR SECRETION OF THE ANTIMALARIAL DRUG CHLOROQUINE

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Introduction: Renal tubular secretion is a major route of elimination of the antimalarial drug chloroquine, but the underlying molecular mechanisms are still unknown. We investigated the involvement of organic cation transporter 2 (OCT2) and multidrug and toxin extrusion protein 1 (MATE1), located in the basolateral and apical membrane of proximal tubular cells, respectively, in transcellular chloroquine transport.

Methods: Chloroquine cellular accumulation and transcellular transport were investigated in single-transfected Madin-Darby canine kidney (MDCK)-OCT2 and MDCK-MATE1 cells and in double-transfected MDCK-OCT2-MATE1 cells grown as polarized monolayers on transwell filters.

Results: Intracellular chloroquine concentrations were significantly lower in the apical compartment from pH 7.8 to 6.0. Addition of trimethoprim (100 μM) to the basal compartment inhibited transcellular chloroquine transport by 87.5% (P < 0.001). Cimetidine inhibited transcellular chloroquine transport in MDCK-MATE1 (IC50 = 16.3 μM) and MDCK-OCT2-MATE1 cells (IC50 = 4.8 μM) in a concentration-dependent manner.

Conclusions: MATE1 mediates the cellular efflux of chloroquine. The pH dependency of MATE1-mediated chloroquine transport is in agreement with the observation that urine acidification increases renal chloroquine elimination. Furthermore, concomitant administration of drugs that inhibit MATE1 may decrease renal chloroquine secretion. OCT2 appears to be of minor importance for chloroquine uptake. However, OCT2 enhances the inhibition of MATE1-mediated chloroquine export by cimetidine, probably because of OCT2-mediated cimetidine uptake.

Topic: Infectious Diseases

Preferred type of Presentation: Poster

Words: 250

P223
PREVALENCE OF COMBINATION OF SPECIFIC ANTIBODIES TO CANDIDA ALBICANS, IGG TO LAMBLIA INTESTINALIS AND IGG TO TRICHOMONAS VAGINALIS IN WOMEN OF REPRODUCTIVE AGE


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Candidiasis of various organs is an important problem of contemporary obstetrics and gynecology. It is assumed that the intestines may be a reservoir of fungi, which results in development of other types of candidiasis under the influence of various exogenous and endogenous factors (Klimko N.N., 2008).

Blood of 530 women of reproductive age was tested for antibodies to Lambitia intestinalis, trichomonads and Candida albicans. Detection of the specific IgG to C. albicans, IgG to V. vaginalis and IgA, IgM, and IgG to Lambitia intestinalis was performed using ELISA (Joint-‘Vector-best’, Novosibirsk).

Depending on the test results, all patients were divided into four groups: women without antibodies to Lambitia intestinalis and to trichomonads (1st group, n = 331), women with antibodies to trichomonads (2nd group, n=90), women with antibodies to Lambitia intestinalis (3rd group, n = 74), and women with combination of antibodies to Lambitia intestinalis and to trichomonads (4th group, n = 35).

The lowest prevalence of IgG to C. albicans (21.5%) was revealed in women of the 1st group. In the other three groups, prevalence of candidiasis markers was significantly higher: 56.7% (2nd group), 43.2% (3rd group) and 51.4% (4th group).

Analysis of titers of the antibodies to C. albicans among patients with positive test results demonstrated that high IgG concentration (1:800 to 1:1600) was detected in 8.5% of women in the group without antibodies to Lambitia intestinalis and trichomonads, in 9.8% of women with antibodies to T. vaginalis, in 25% of women with antibodies to L. intestinalis, and in 22.2% of patients with combination of antibodies to Lambitia intestinalis and to trichomonads.

Thus, the highest prevalence of increased IgG titers to C. albicans has been detected in the groups with signs of compromised colonial resistance of the intestines. Trichomonads and lambia operate both locally and systemically; they not only allocate somatic antigens and metabolites and absorb nutrients, but also create favorable conditions for activation of opportunistic infections and aggravate dysbacteriosis in the intestines and in the genital organs.
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QUALITY CONTROL OF 22 BATCHES OF IVHEBEX® PRODUCED IN 2010, THE INTRAVENOUS HEPATITIS B IMMUNOGLOBULIN OF LFB BIOMEDICAMENTS
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Introduction: Intravenous Hepatitis B immunoglobulin (HBIG; IVHEBEX®) is a specific immunoglobulin produced from human hyperimmune plasma and marketed in France since 1995 for long-term prophylaxis against the recurrence of liver hepatitis B virus (HBV) infection in HBV-infected patients formerly having undergone liver transplantation. Its manufacturing process includes ethanolic precipitation and pH4/pepsin treatment.

Method and results: We present here the results of the quality controls performed on 22 commercial batches produced in 2010. The 22 batches are compliant with the specifications described in the European Pharmacopoeia and in the marketing authorisation dossier in France. Low variation coefficients are fully reproducible on a long-term basis, and ensure the reproducibility of the quality and the security of this product.

P225
ABCB1 GENE POLYMORPHISMS AND NEUROPSYCHIATRIC ADVERSE EVENTS IN OSELTAMIVIR-TREATED CHILDREN DURING INFLUENZA H1N1/09 PANDEMIA
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Introduction: Oseltamivir has been recently associated with neuropsychiatric adverse events (NPAE) in children. Oseltamivir, but not its carboxylate active metabolite, is a substrate of P-glycoprotein (P-gp). We conducted a prospective cohort study to evaluate the impact of P-gp polymorphism on the incidence of NPAE in oseltamivir-treated children during the H1N1 pandemic.

Methods: This study was conducted in the department of paediatrics of the University Hospitals of Geneva, Switzerland, between October 1st, 2009 and January 31st, 2010. All newborn to 18 year-old patients presenting with a flu-like illness were eligible for inclusion. Adverse events were systematically recorded by paediatricians and/or parents using a diary card, with a 30 days follow-up. The causality assessment of oseltamivir in NPAE was performed by two clinical pharmacologists. After informed consent, enrolled patients were genotyped for ABCB1 C3435T and G2677T/A polymorphisms.

Results: Among the 42 H1N1-infected, oseltamivir-treated children being genotyped for ABCB1 C3435T and G2677T/A variants, 36% (15) presented NPAE possibly related to oseltamivir. The frequency of NPAE displayed a ‘genotype-trend effect’ with the mutant and the wild type subgroups at the two fare ends. Sixty-seven percent of the 2677TT-3435TT individuals (mutant homozygotes) presented NPAE compared to 39% of heterozygotes and 11% of the 2677GG-3435CC individuals (wild-type homozygotes) (NS). A pairwise comparison between 2677TT-3435TT (mutant homozygotes) and 2677GG-3435CC (wild-type homozygotes) individuals showed a trend toward statistical significance (P = 0.054).

Conclusions: Our results suggest that the 2677TT-3435TT haplotype might increase the patient vulnerability to NPAE, maybe as a result of enhanced permeability of the blood-brain-barrier to oseltamivir.

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PHARMACOKINETICS OF GENTAMICIN IN EXPERIMENTAL SEPSIS
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We investigated the influence of covariates generated by a rat sepsis model on gentamicin (Ge) pharmacokinetics. Endotoxemia was induced by i.v. administration of 1 mg/kg lipopolysaccharide (Pseudomonas aeruginosa) in combination with 15 µg/kg recombinant mice interleukin-2 (LPSI). Ge i.v. bolus (3 mg/kg) was followed by i.v. 170-min Ge infusion (0.09 mg/kg/min) started 10 min later. Experimental groups (n = 7/each) included controls infused with saline only (group 1), rats infused with saline followed by Ge (group 2), rats given LPSI as an i.v. bolus and i.v. saline in continuous infusion (group 3), and rats given LPSI followed by Ge (group 4). Blood sampling: at baseline, during Ge treatment and at 420 min of experiment, when rats were euthanized and target organs removed for determination of injury. Endotoxemia induced microvascular leakage, decrease in glomerular filtration rate, tubular dysfunction, and lactatemia (group 1 vs. 3, examination by clinical chemistry). Histopathology proved pulmonary changes (leakage, alveolar wall oedema, inflammatory cells, and erythrocytes in the alveoli). Spleen showed a higher amount of erythrocytes infiltrated to the white pulp. Changes of Ge pharmacokinetics in endotoxemia (group 4 vs. 2) were as follows: larger Vd: 0.343 ± 0.117 vs. 0.224 ± 0.072 l/kg (P > 0.02), decreased renal clearance: 2.18 ± 1.55 vs. 3.81 ± 1.39 ml/min/kg (P > 0.03), while total plasma clearance was comparable 5.95 ± 2.04 vs. 5.78 ± 0.20 ml/min/kg, with rising interindividual variability in endotoxemia (34.3% vs. 2.9%, respectively, CV[%=] σ/μ). T1/2 was prolonged: 45.43 ± 12.15 vs. 30.58 ± 1.16 min (P = 0.007). In endotoxemia, Ge dosage should be kinetically guided.
Method: A case of a 10 month old with acute malaria treated with artemether-lumefantrine. Following a 2 day episode of fever and restlessness, a 10 month old child was placed on artemether-lumefantrine combination for 3 days. Fever was unresolved and the patient was also diagnosed with tonsillitis and acute diarrhoea as well as presenting with a low PCV. Antibiotic therapy was instituted alongside hematinics and rehydration therapy. Temperature failed to subside after 2 days with declining PCV value. Malaria parasite screen was conducted again and found to be positive. FBC, urea and creatinine tests were also conducted and were of abnormal values. Physician impression was to use the old chloroquine regimen. The patient was commenced on chloroquine immediately.

Results: Temperature subsided within few hours of instituting therapy and patient recovered to full activity within a few days. The currently reported case thus presents a possibility of re introduction of older drugs with new sensitivity profile following switch to other drugs.

Conclusion: Chloroquine resistance in regions of malaria may need re evaluation as the trend of resistance may change susceptibility patterns across the globe.

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GENETIC POLYMORPHISM OF CYTOCHROME P4502B6 IS NOT SIGNIFICANTLY ASSOCIATED WITH NEVIRAPINE-INDUCED RASH IN MALAYSIAN POPULATION

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Introduction: Nevirapine-induced rash is widely observed after starting therapy among Malaysian HIV-1 infected patients with incidence higher than 21%. Nevirapine (NVP) is metabolized by Cytochrome P450 P450 system enzymes like CYP2B6.

Patients and methods: Only diagnosed HIV-1 patients received NVP as one agent of their antiretroviral treatment were recruited in this study from three Malaysian Hospitals, they are, Hospital Sungai Buloh/Selangor, Hospital Sultanhah/Bahiyah/Alor Setar, and Pulau Pinang General Hospital/Pulau Pinang, based on predefined inclusion criteria. Patient DNA was extracted from peripheral blood samples by (QiAmp DNA Mini Kit, Qiagen, Germany). Applying nested Polymerase Chain Reaction (PCR) Method to investigate three SNPs in CYP2B6 Gene, they are: 64C>T, 516G>T, and 785A>G leading to three different alleles, these alleles are: CYP2B6*2A, CYP2B6*4A, and CYP2B6*6A.

Results: Out of this study patients samples pool, forty samples were selected randomly for genotyping. Among them, 15 (37.5%) discontinued NVP because of NVP adverse reaction. Levels of Bacteroides fragilis and LCA in the feces correlated with the Cyp3a expression level.

Conclusion: Nevirapine-Induced rash is widely observed after starting therapy among Malaysian HIV-1 infected patients with incidence higher than 21%. Nevirapine (NVP) is metabolized by Cytochrome P450 system enzymes like CYP2B6.

Results and Conclusion: The mRNA expression level of hepatic Cyp3a11, LCA, and LCA-producing bacterial (Bacteroides fragilis) DNA, in the feces on 1, 2, 3, 4, and 5 days after the CPX administration to mice (200 mg/kg/day), and 7 and 14 days after discontinuing administration were measured.

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COMPARISON OF THE PHARMACOKINETICS OF CEFTRIAXONE AND LEVOFLOXACIN IN SURGICAL PATIENTS

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Patients undergoing hip or knee replacement therapy are routinely pre-treated with antibiotics before they enter the theatre. This treatment intends to reduce the incidence and severity of peri- or post-surgical infections. It is controversial which antibiotic is the treatment of choice for this purpose. We wanted to study the plasma and bone kinetics of ceftriaxone and levofloxacin in orthopedic patients in order to see whether in the spongiosa and the corticalis the bones of these patients sufficiently high concentrations of the antibiotic are obtained in order to inhibit usual hospital infections. Therefore, patients (22 or 32, respectively) undergoing routine surgery were treated with ceftriaxone 2 g intravenously as a bolus immediately prior to operation. Alternatively, patients were given 500 mg levofloxacin intravenously for 60 min as a constant infusion. Plasma samples were withdrawn before and at three time points after drug infusion. After replacement of the bones, extracts from spongiosa or corticalis were obtained. Samples were subjected to standard extractions procedures and ceftriaxone or levofloxacin, which are hardly metabolized, were quantified using high performance liquid chromatography (HPLC). The kinetics of ceftriaxone and levofloxacin distribution into bone were analyzed using a population approach (ADAPT5). We noted, that the corticalis contained significantly higher levels of ceftriaxone or levofloxacin (15.7 mg/l or 26.7 mg/l) than the spongiosa (10.8 mg/l or 16.6 mg/l, n = 22–32). The minimal inhibitory concentration (MIC) of ceftriaxone or levofloxacin for susceptible staphylococci amounts to 0.2–2 mg/l or 0.03–1 mg/l. It is concluded that the concentration of levofloxacin and ceftriaxone in spongiosa and corticalis should be adequate to protect the patients against the usual nosocomial infections.

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THE ROLE OF SECONDARY BILE ACID ON A DECREASE IN HEPATIC CYTOCHROME P450 3A EXPRESSION LEVEL BY CIPROFLOXACIN

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Introduction: We found that ciprofloxacin (CPX) administration caused decreased enterobacteria-produced lithocholic acid (LCA), resulting in the decrease in hepatic Cytochrome P450 3A (Cyp3a) expression level. This study examined the relation between the CPX administration period and hepatic Cyp3a expression, and the role of secondary bile acid other than LCA on Cyp3a expression.

Materials and Methods: The levels of mRNA expression of hepatic Cyp3a11, LCA, and LCA-producing bacterial (Bacteroides fragilis) DNA, in the feces on 1, 2, 3, 4, and 5 days after the CPX administration to mice (200 mg/kg/day), and 7 and 14 days after discontinuing administration were measured.

Results and Conclusion: The mRNA expression level of hepatic Cyp3a11 decreased from day 1 of CPX administration caused decreased enterobacteria-produced lithocholic acid (LCA), resulting in the decrease in hepatic Cytochrome P450 3A (Cyp3a) expression level. The CYP3A4 transcriptional activation potential of secondary bile acids such as LCA, deoxycholic acid (DCA), taurocholic acid (TCA), and taurodeoxycholic acid (TDCa) was examined using luciferase assay. The mRNA expression level of hepatic Cyp3a11 was measured for 5 days after administrating CPX alone or CPX with secondary bile acid.

Results and Conclusion: The mRNA expression level of hepatic Cyp3a decreased from day 1 of CPX administration and recovered upon discontinuation. Levels of Bacteroides fragilis and LCA in the feces correlated with the Cyp3a expression level. CYP3A4 transcriptional activities were augmented in the presence of all secondary bile acids.

The decrease of the mRNA expression level of CYP3A11 by CPX was improved by co-administration of LCA or DCA, while TCA and TDCa exerted no change. These results show that the decrease of the Cyp3a expression level by CPX was caused mainly by the decreased LCA level and recovered 14 days after discontinuing administration.
INNOVATIVE BIOSIMILAR PRODUCTS

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NEW EXPERIMENTAL DATA ON THE BIOAVAILABILITY AND BLOOD BRAIN BARRIER PENETRATION OF A SYSTEMICALLY ADMINISTERED SILICA NANOPARTICLES
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The blood brain barrier penetration and availability of silica nanoparticles (SNP) in mice was studied in order to determine the possible uses as a drug carrier.

Methods: Fluorescent silica nanoparticles (SNP + Glucose) were administered i.p. A different group of six mice received a similar volume of glucose solution as control. The animals were sacrificed at 1 h and samples of brain were harvested and prepared for analysis by fluorescence confocal microscopy. Coronal sections were cut using a freezing microtome (CM 1850; Leica Microsystems, Germany). Sections were collected, mounted on slides and examined in a Laser Scanning Confocal Microscope TCS SPE (Leica Microsystems, Germany), 480 nm excitation and 610–630 nm emission. For positive control, the injected glucose SNP solutions were also examined under microscope.

Results: Compared to the control sections, the sections from SNP treated animals displayed dotted fluorescence of significant magnitude. Therefore, following i.p. administration, our new SNP penetrated the blood brain barrier. The results support the idea of using our SNP as a container for modular drug delivery system with usage in pain control drugs.

Conclusions: Innovative painkillers of the future require targeted carriers for improved analgesia and patient compliance. The tested SNP is a possible candidate as a delivery system for such drugs.

Acknowledgements: This work was supported by a Romanian Education and Research Ministry grant, PNCDI IDEI (no. 1734/2008).

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ETHICAL CONSIDERATIONS IN DEVELOPING BIOSIMILARS
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Introduction: Biosimilars offer limited advantage to patients, and yet, the risk of adverse reactions is similar to novel compounds. Not undermining the value of biosimilars: Their lower price makes them more accessible, improved use of current technologies can contribute to purity and safety, but essentially, they are at best only slightly improved copies. This places responsibility on the developers and regulators; on one hand to ensure high level of safety monitoring and on the other hand to contribute to science in return for the risks and discomforts endured by the study subjects.

Recommendations: If no significant advantage is expected, then no additional risk can be tolerated. In clinical trials this means that an appropriate real-time safety monitoring is established, supplemented by an external independent Data Safety Monitoring Board (DSMB). Evaluation of immunogenicity is of primary importance. Most drastic manifestations are immediate hypersensitivity reaction, such as anaphylaxis, but lack of efficacy indicating potential development of neutralizing antibodies is no less important. In addition to ensuring safety, a biosimilar development plan should consider modalities to provide benefit by improving treatment for the target patient population. This can be through evaluation of pharmacogenetic parameters, biomarkers, evaluation of subpopulations likely to benefit from treatment, etc.

Conclusions: Scientific and technological progress dictates the need for development of medical ethics. The Helsinki Declaration underwent several updates to meet the contemporary standards. Biosimilars, similarly to other advanced therapies, should adhere to specific deontological principles.

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PROSPECTIVE EVALUATION OF THE DOSSING REGIMEN OF VANCOMYCIN IN CHILDREN OF DIFFERENT WEIGHTS
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Introduction: The prevalence of obesity in children has steadily risen during recent years in developed countries. Obesity in children is associated with increased use of health care system resources. Limited data is available regarding the pharmacokinetics, pharmacodynamics and optimal dosing of most antimicrobials for obese patients, especially in the pediatric population. An accurate estimation of volume of distribution (Vd) and clearance (CL) is essential for determining appropriate dosing regimen. Recent guidelines suggested increased trough levels of vancomycin in order to achieve efficacy and prevention of the development of resistant strains. This may be limited by nephrotoxic and otoxic adverse effects. This study was designed prospectively to investigate the pharmacokinetic parameters of vancomycin among obese compared to normal and overweight children and adherence to current recent guidelines suggesting higher trough levels for severe infections.

Patients and Methods: All pediatric patients in the Meyer Pediatric Hospital receiving vancomycin, (standard dosing regimen 20 mg/kg twice daily), between March 2010 and February 2011 were included. Patients were divided into three groups, overweight (Group O), normal weight (Group N) and underweight (Group U). Guidelines for vancomycin monitoring for children are lacking, hence adequacy was defined as for adults e.g. trough level beyond 10 mg/l and AUC/MIC beyond 400. Based on pharmacokinetic parameters, the optimal dosage was calculated.

Results: Eighty pairs of levels (trough and peak) were taken from 53 children. Trough range for the standard regimen was 0.24–12.62 mg/l, peak range 5.75–45.66 mg/l. Mean trough for all children 3.36 ± 2.58 mg/l (group U 4.16 ± 3.55 mg/l, group N 3.54 ± 2.51 mg/l, group O 3.06 ± 1.86 mg/l). Mean peak 21.36 ± 8.74 mg/l (group U 22.33 ± 10.42 mg/l, group N 20.87 ± 7.84 mg/l, group O 21.67 ± 7.87 mg/l). Only 3% of the trough levels were in the therapeutic range (8% of group U, 3% of group N and none (0%) of group O). Mean value of AUC/MIC was 149 ± 98; none of the children had a value above 400. Mean elimination t1/2 was 3.42 ± 1.26 h (group U 3.55 ± 1.19, group N 3.65 ± 1.4, group O 3.3 ± 1.7). Mean CL was 0.15 ± 0.08 l/h/kg (group U 0.16 ± 0.09 l/h/kg, group N 0.16 ± 0.09 l/h/kg, group O 0.13 ± 0.05 l/h/kg). Mean Vd was 0.72 ± 0.37 l/kg (group U 0.78 ± 0.51 l/kg, group N 0.79 ± 0.32 l/kg, group O 0.62 ± 0.34 l/kg). In order to reach optimal blood levels (trough beyond 10 mg/l) a dosing regimen of 13 mg/kg, given over 1 h, four times daily is needed.

Conclusion: Based on pharmacokinetic parameters the dosing regimen should be reevaluated.
Children of all weight groups need more frequent and higher dosing of vancomycin in order to achieve optimal blood levels. Personalized medicine is the ideal way to achieve desired outcomes.

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INTRA VENOUS PARACETAMOL PHARMACOKINETICS IN NEONATES: WEIGHT OUTWEIGHTS AGE
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Background: The aim was to describe intravenous (IV) paracetamol pharmacokinetics, to determine major covariates and to suggest a dosing regimen for neonates 28–44 weeks postmenstrual age (PMA).

Methods: A population pharmacokinetic analysis of paracetamol time-concentration profiles (943 observations) from 158 neonates [PMA: 27–45 weeks] was undertaken using non-linear mixed effects models (NONMEM). Data from three published studies were pooled with newly collected time-concentration profiles during repeated IV paracetamol administration in neonates (PARANEO study) (12,3).

Results: A two-compartment (central, peripheral) linear disposition model was used. Population parameter estimates (between subject variability, %) were central volume (V1) 51.9 (21.6%) L.70 kg, peripheral volume of distribution (V2) 22.7 L.70 kg, clearance (CL) 5 (40%) L/h.70 kg and inter-compartment clearance (Q) 16.2 L/h.70 kg. Covariate information predicts 60.9% of the clearance variance. Weight was used to predict patient size and this was the major covariate contributing 57.5% of variance. Clearance expressed as mg/kg/h increases only slightly with PMA (0.138 L/kg/h at 28 to 0.167 L/kg/h at 44 weeks PMA), and contributes to only 2.2% of variance. High unconjugated bilirubin levels only contributed an additional 1.2% of variance.

Conclusions: Patient size (predicted by weight) is the major covariate of clearance variance of IV paracetamol. Using the estimates obtained, a mean paracetamol serum concentration of 11 mg/l is predicted in neonates 28–44 weeks PMA given a standard dose of IV paracetamol 10 mg/kg 6 h. Since there remains only limited data about the safety of this drug in neonates. Continued surveillance remains essential.

References:

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AZATHIOPRINE DOSING IN CHILDREN WITH INFLAMMATORY BOWEL DISEASE (IBD)
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Introduction: Azathioprine (Aza) is effective for the induction and maintenance of remission for pediatric IBD patients. 6-TGN levels of 250–450 pmol/8.108 RBC are considered as therapeutic goal. The standard dose of Aza is 2–2.5 mg/kg/day. Although dose escalation was found associated with risk of adverse events in adults IBD, recent studies suggest an increased dosing in pediatric IBD patients. The aim of this study is to assess the effect of aza dose on efficacy and safety in pediatric IBD patients.

Methods: Data from 62 children treated by Aza in monotherapy for at least 3 months were collected retrospectively. Patients were divided into three groups according to Aza dosage: under 2 mg/kg/d, 2–2.5 mg/kg/d (standard dosing) and above 2.5 mg/kg/d. Patients were considered in remission if their PDCAI was >10. Blood cell counts and liver function tests were determined to evaluate adverse effects. 6-TGN and 6-MeMPN concentrations were measured in RBC by HPLC.

Results and discussion: 89.6% of patients receiving an AZA dose >2 mg/kg/d achieved clinical remission compared to 71.9% in children treated by a dose above 2.5 mg/kg/d (P = 0.031). Aza dose was positively related to RBC 6-TGN values (r = 0.172, P = 0.021) as well as 6-MeMPN levels (r = 0.305, P < 0.001). There is a tendency for the occurrence of lymphopenia in patients treated with AZA dose above 2.5 mg/kg/d compared with those receiving AZA dose under 2 mg/kg/d or the standard dosing, (16.7% vs. 13.1 and 13.7% respectively). Likewise, 9.7% of children experienced leukopenia in the group treated with AZA dose above 2.5 mg/kg/d compared to 7.7% and 7.8% in the group under 2 mg/kg/d and standard dosing respectively. Patients with hematologic abnormalities had 6-TGN levels higher than 450 pmol/8.108 RBC. An increase in levels of liver and pancreatic enzymes was also observed in children receiving Aza dose above 2.5 mg/kg/d compared to other groups.

Conclusion: These preliminary observations suggest that Aza dose above 2.5 mg/kg/d in pediatric IBD patients may be associated with substantial risk of dose-dependant adverse events without improvement of clinical remission.

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C. TRACHOMATIS AS A CAUSE OF FREQUENT EXACERBATIONS OF CHRONIC PYELONEPHRITIS IN YOUNG GIRLS
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Aim: To determine the place of C. trachomatis in exacerbations of chronic pyelonephritis in young girls.

Materials and Methods: A total of 40 female patients aged 3–14 years with exacerbations of chronic pyelonephritis were observed during 2000–2005 years in pediatric hospital. All patients were done urine culture with antibiotic susceptibility testing according. Also all girls were examined by gynecologist. There were swabbed the urethra and conjunctiva. The swabs were analyzed by direct fluorescence. All patients were administered the adequate antibacterial therapy. Azithromycin in dose of 10 mg/kg for 14 days was added to the patients who were positive for C. trachomatis.

Results: Annual exacerbation of chronic pyelonephritis was observed in 25% (10/40) patients (group 1) and 3–4 episodes per year - in 75% (30/ 40) patients (group 2). Of 90% urine cultures of all subjects had growth of E. coli with a count of more than 105 CFU/ml. All these pathogens were susceptible to aminopenicillines, cephalosporines and aminoglycosides. There were isolated other Enterobacteriaceae in other patients (10%). At each hospitalization the girls of second group [66.6% (20/30)] had vulvovaginitis which remained during the treatment of exacerbation of chronic pyelonephritis. All these patients with vulvovaginitis had positive urethra smears for C. trachomatis, but 75% of these girls had positive conjunctiva smears for this one. All control swabs were negative in one and 3 months after treatment completion. The patients of second group had no relapses of pyelonephritis for the next 3 years.

Conclusions: The frequent relapses of chronic pyelonephritis in children may be related to chronic C. trachomatis infection. All children with recurrent pyelonephritis should be examined for the presence of C. trachomatis.
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DRUG INTERACTION BETWEEN LAMOTRIGINE AND VALPROIC ACID USED AT DELIVERY AND DURING LACTATION – A CASE REPORT
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Introduction: Lamotrigine and valproic acid have become two of the three major drugs using in the treatment of pregnant women with epilepsy. Concomitant administration of valproic acid significantly decreases lamotrigine clearance at delivery by about 65%. Regular therapeutic drug monitoring of antiepileptic drugs during pregnancy and postpartum is recommend. In our case report we show a woman treated by combination of lamotrigine and valproic acid at delivery and during first postnatal month. We analyzed maternal clearance, transport through the placenta and to maternal milk and exposure in breastfed infant of both antiepileptic drugs.

Methods: The patient was treated with combination of lamotrigine and valproic acid. Milk and blood samples from the mother and her breastfed infant were collected at delivery and during first postnatal month. Maternal serum levels were used for the estimation of apparent oral clearance. Paired milk and maternal, infant and maternal and infant milk concentrations were used for estimation of the ratios. Valproic acid concentrations were measured by gas chromatography and lamotrigine concentrations by high-performance liquid chromatography.

Results: The infant lamotrigine concentration (13.6 mg/l) was found at delivery equal to the maternal (12.3 mg/l). The infant valproic acid concentration (43.4 mg/l) was measured at delivery by about 40% higher than in the mother (31.7 mg/l). Four days after delivery was found the infant lamotrigine concentration (12.7 mg/l) similar to maternal (11.7 mg/l) and the infant valproic acid concentration (13.5 mg/l) as third compared to maternal (37.8 mg/l). After that were measured the infant lamotrigine concentrations (6.7–6.9 mg/l) approximately half of the maternal levels (14.0–15.2 mg/l). The infant valproic acid levels were under detectable limit for all the time. Maternal lamotrigine and valproic acid clearances were similar all the time.

Conclusion: In our case report we showed interaction between lamotrigine and valproic acid. Main reasons of relatively high breastfed infant lamotrigine concentrations are treatment by high maternal lamotrigine dose in combination with inhibition effect of valproic acid, transport to maternal milk and immature metabolizing pathway of breastfed infant. Therapeutic monitoring of infant lamotrigine concentrations appears to be the most relevant method for analysis of lamotrigine exposure in breastfed infants.

P239
SERUM LEVELS OF LAMOTRIGINE IN BREASTFEEDING MOTHERS, MATERNAL MILK AND NURSED INFANTS
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Introduction: The clearance of lamotrigine quickly returns close to pre-pregnancy levels in the first 1–2 weeks after delivery leading to symptoms of toxicity in women whose dose has been increased during pregnancy. Therefore frequent lamotrigine level monitoring is necessary not only during pregnancy but also after delivery. Lamotrigine excretion into breast milk was reported, however the data on the lamotrigine transfer into breast milk and the risk of exposure to the breastfed infants remain sparse and only a limited number of studies have actually measured infant blood levels. In our study we followed up lamotrigine transport from breastfeeding mothers to the maternal milk and their breastfed infants and analyzed maternal lamotrigine clearance in monotherapy 6–10 days after delivery in comparison with the time of delivery.

Methods: Maternal, infant and milk concentrations were analyzed 6–10 days after delivery in a cohort of 21 women between the years 2001–2009. The request forms for routine therapeutic drug monitoring were used as the data source. Maternal concentrations were used for the estimation of apparent oral clearance. Paired milk and maternal and paired infant and maternal concentrations were used for the estimation of the milk/maternal serum and the infant/maternal serum concentration ratios. Paired values of maternal clearance were used for the estimation of changes in lamotrigine clearance between the time of delivery and approximately 1 week after delivery. Lamotrigine concentrations were measured by high-performance liquid chromatography. Statistical tests were performed using GraphPad Prism version 5.00 for Windows.

Results: The lamotrigine concentrations varied from 1 to 12.6 mg/l in the maternal serum, from 0.0 to 3.8 mg/l in the infant serum and between 0.4 and 4.5 mg/l in the milk. The milk/maternal serum concentration ratios ranged from 0.21 to 0.74 and the infant/maternal serum concentration ratios from 0.00 to 0.74. Highly significant correlations were found between the milk and the maternal serum levels, the infant and the maternal serum levels and the infant and the milk lamotrigine levels. Values of the infant/maternal serum concentration ratios were calculated significantly lower than values of the milk/maternal serum concentration ratios. Significant correlation was not found between the infant/maternal serum concentration ratios and the milk/maternal serum concentration ratios. Maternal lamotrigine clearance decreased by about 33% after delivery.

Conclusions: Our data showed the interindividual variability of the infant/maternal serum lamotrigine concentration ratios caused probably by the different activity of the infant metabolizing enzymes UGT1A4 and 2B7 in conjunction with the interindividual variability in the milk/maternal serum concentration ratios. The potential adverse effects of lamotrigine should be associated with the higher concentrations in breastfed infants.

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MONITORING OF VALPROIC ACID CONCENTRATIONS IN BREASTFEEDING MOTHERS, MATERNAL MILK AND NURSED INFANTS
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Introduction: Only carbamazepine and lamotrigine are prescribed during pregnancy more often than valproic acid. Regular therapeutic monitoring of valproic acid during pregnancy and postpartum is recommended but the data on the valproic acid transfer to the milk and the risk of exposure to the breastfed infants remain sparse and only a limited number of studies have actually measured the infant blood levels. In our study we followed up valproic acid transport from the breastfeed-ing mothers to the maternal milk and their breastfed infants and analyzed the influence of co-medication with enzyme-inducing antiepileptic drugs.

Methods: Maternal, infant and milk concentrations were analyzed 6–10 days after delivery in a cohort of 27 women between the years 1991–2009. The request forms for routine therapeutic drug monitoring were used as the data source. Paired milk and maternal, paired infant and maternal and paired infant and milk concentrations were used for estimation of the milk/maternal serum, the infant/maternal serum and the infant serum/milk concentration ratios. Valproic acid concentrations were measured by gas chromatography. Statistical tests were performed using GraphPad Prism version 5.00 for Windows.

Results: The valproic acid concentrations varied from 17.0 to 69.0 mg/l in the maternal serum, from 0.0 to 17.5 mg/l in the infant serum and between 0.0 and 32.8 mg/l in the milk. The milk/maternal serum concentration ratios ranged from 0.09 to 0.78 for lamotrigine, the milk valproic acid concentrations were under detectable limit for all the time. The milk/maternity serum valproic acid concentrations were under detectable limit for all the time. The milk/maternal serum valproic acid concentrations were similar all the time.

Conclusions: In our case report we showed interaction between lamotrigine and valproic acid. Main reasons of relatively high breastfed infant valproic acid concentrations are treatment by high maternal valproic acid dose in combination with inhibition effect of valproic acid, transport to maternal milk and immature metabolizing pathway of breastfed infant. Therapeutic monitoring of infant valproic acid concentrations appears to be the most relevant method for analysis of valproic acid exposure in breastfed infants.
observed between valproic acid monotherapy + non-enzyme-inducing antiepileptic drugs and valproic acid + enzyme-inducing antiepileptic drugs subgroups. Seventeen percent of values of the milk/maternal serum concentration ratios were found higher than reported maximal value 0.10.

Conclusions: Our data showed the interindividual variability of the infant/maternal serum valproic acid concentration ratios caused probably by the different activity of the infant metabolizing enzymes CYP2C9, CYP2C19 and UGT2B7 in conjunction with the interindividual variability in the milk/maternal serum concentration ratios. The potential adverse effects of valproic acid could be associated with the higher concentrations in breastfed infants.

P242
COMPARATIVE ASSESSMENT OF THE GROWTH CURVES IN EU AND US
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Introduction: Allometric scaling may be applied along with other physiologically based adjustments to predict paediatric pharmacokinetics using demographic variables such as weight and BMI. Validated growth curves from several EU countries are available including Germany, Italy, Spain, and Sweden. For modeling purposes it may be advantageous to have a centralized database that is relevant across the EU continent. The objective of this analysis is to compare available growth curves across EU countries to those of the CDC and the WHO databases.

Methods: Published growth curves from EU countries, CDC and WHO were used to extract the age-matched body weight (WT) and Body Mass Index (BMI) in boys and girls between the ages of 0 and 18 years. The WT was back calculated using the height and BMI if unavailable. The median values were compared across the following age groups where available:
Infants and Toddlers (28 day to 23 months)
Children (2–11 years)
Adolescent (12–18 years)

Results: The aged-matched median body weights across EU countries, WHO, and CDC scales appear comparable, especially when considering the associated variability demonstrated by the 5th and 95th percentile curves. The median values were within ±12% of the CDC database. A trend of higher WT was observed in German and Italian boys relative to CDC. The maximum difference of 12% occurred at age 9.5 in Italian boys.

Conclusion: The current analysis suggests that the CDC database can be adequately used for allometric scaling modeling in EU submissions.

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MONITORING OF VALPROIC ACID CONCENTRATIONS DURING DELIVERY IN MOTHERS AND THEIR INFANTS
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Introduction: Valproic acid has become one of the three major drugs in the treatment of pregnant women with epilepsy and its regular therapeutic monitoring during pregnancy is recommended. Literature data about intrauterine growth retardation associated with the maternal use of valproic acid are controversial and relationship between valproic acid concentrations and birth weight (and length) has not been analyzed. In our study we followed up valproic acid transport through the placenta and demonstrated maternal and umbilical cord serum levels, its ratio, maternal clearance and influence of co-medication with enzyme-inductive antiepileptic drugs. We analyzed the relationship between birth weight (and length) and daily dose, dose related to the body weight, and maternal and infant valproic acid concentrations.

Methods: Maternal and umbilical cord serum levels were analyzed during delivery in a cohort of 56 women between the years 1991–2009. The request forms for routine therapeutic drug monitoring were used as the data source. Maternal concentrations were used for the estimation of apparent oral clearance and paired infant and maternal concentrations for estimation of the infant (umbilical cord)/maternal serum concentration ratios. Valproic acid concentrations were measured by gas chromatography. Statistical tests were performed using GraphPad Prism version 5.00 for Windows.

Results: The valproic acid concentrations varied from 5.3 to 59.5 mg/l in the maternal serum and between 5.4 and 72.1 mg/l in the umbilical cord serum. The infant/maternal serum concentration ratios ranged from 0.64 to 2.49 (mean 1.47). Concomitant medication with enzyme-inducers increased significantly clearance of valproic acid by about 40%. Highly significant correlations were found between maternal and umbilical cord valproic acid serum levels, both in monotherapy (and/or combination with lamotrigine) and in combination with enzyme-inducers. The infant/maternal valproic acid concentration ratios correlated inversely with the maternal valproic acid levels. Significant inversely correlations were found between birth weight and maternal valproic acid concentrations, and birth length and maternal and infant valproic acid concentrations.

Conclusions: Our data from the large cohort showed the interindividual variability of umbilical cord/maternal serum concentration ratios of valproic acid caused probably by the different activity of the placental metabolizing enzyme UGT2B7 and active transport system associated with genetic polymorphism. The potential teratogenic effect of valproic acid seems to be associated with the higher concentrations in the fetus but not with the maternal dose.

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SERUM LEVELS OF LAMOTRIGINE IN BREASTFEEDING MOTHERS, MATERNAL MILK AND NURSED INFANTS
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Introduction: The clearance of lamotrigine quickly returns close to pre-pregnancy levels in the first 1–2 weeks after delivery leading to symptoms of toxicity in women whose dose has been increased during pregnancy. Therefore frequent lamotrigine level monitoring is necessary not only during pregnancy but also after delivery. Lamotrigine excretion into breast milk was reported, however the data on the lamotrigine transfer into breast milk and the risk of exposure to the breastfed infants remain sparse and only a limited number of studies have actually measured infant blood levels. In our study we followed up lamotrigine transport from breastfeeding mothers to the maternal milk and their breastfed infants and analyzed maternal lamotrigine clearance in monotherapy 6–10 days after delivery in comparison with the time of delivery.

Methods: Maternal, infant and milk concentrations were analyzed 6–10 days after delivery in a cohort of 21 women between the years 2001–2009. The request forms for routine therapeutic drug monitoring were used as the data source. Maternal concentrations were used for the estimation of apparent oral clearance. Paired milk and maternal and paired infant and maternal concentrations were used for the estimation of the milk/maternal serum and the infant/maternal serum concentration ratios. Paired values of maternal clearance were used for the estimation of changes in lamotrigine clearance between the time of delivery and approximately 1 week after delivery. Lamotrigine concentrations were measured by high-performance liquid chromatography. Statistical tests were performed using GraphPad Prism version 5.00 for Windows.

Results: The lamotrigine concentrations varied from 1.1 to 12.6 mg/l in the maternal serum, from 0.0 to 3.8 mg/l in the infant serum and between 0.4 and 4.5 mg/l in the milk. The milk/maternal serum concentration ratios ranged from 0.21 to 0.74 and the infant/maternal serum concentration ratios from 0.00 to 0.74. Highly significant correlations were found between the milk and the maternal serum levels, the infant and the mater-
nal serum levels and the infant serum and the milk lamotrigine levels. Values of the infant/maternal serum concentration ratios were calculated significantly lower than values of the milk/maternal serum concentration ratios. Significant correlation was not found between the infant/maternal serum concentration ratios and the milk/maternal serum concentration ratios. Maternal lamotrigine clearance decreased by about 33% after delivery.

**Conclusions:** Our data showed the interindividual variability of the infant/maternal serum lamotrigine concentration ratios caused probably by the different activity of the infant metabolizing enzymes UGT1A4 and 2B7 in conjunction with the interindividual variability in the milk/maternal serum concentration ratios. The potential adverse effects of lamotrigine should be associated with the higher concentrations in breast-fed infants.
PHARMACOEPIDEMIOLOGY

P245
OMEPRAZOLE PRESCRIBING AND ITS COST IMPACT IN OUTPATIENT CLINICS AT SULTAN QABOOS UNIVERSITY HOSPITAL
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Introduction: In spite of a number of local and international guidelines, use of omeprazole in hospitals is increasing rapidly as is the expenditure. The aim of the study was to report indications for omeprazole prescribing and compare prescribing duration with local and NICE guidelines. The study also determined the total consumption and cost of omeprazole prescribed at Sultan Qaboos University Hospital (SQUH).

Methods: Retrospective observational study performed over a 6-month period in outpatients who attended gastroenterology and non-gastroenterology clinics at SQUH. The total number of prescriptions dispensed during the study period was 2622. After excluding children and adults under 18 years of age, and patients with a history of upper gastrointestinal bleeding, the sample size was 1662. From this pool, a random sample of 30% was taken to obtain a sample size of 499 which was the study cohort.

Results: Thirty one percent of the prescriptions were from the gastroenterology outpatient clinic while 69% were from other clinics. In 27% of the patients it was prescribed for those who were prescribed drugs associated with ulcers. Ten percent of the patients had prescriptions for gastritis, 25% for dyspepsia, 4% for peptic ulcer disease, and 16% for gastroesophageal reflux disease (GERD), 6% for the eradication of Helicobacter pylori and in 12% there was no documentation. Eighteen percent of the cohort had over prescribing (20% of females and 14% of males). Multivariate logistic regression model showed that males were 38% less likely to have over prescribing compared to females. Furthermore, gastroenterology clinics were 96% more likely to have over prescribing than non-gastroenterology clinics. With respect to age, the model indicated that, generally, the odds of having over prescribing increased with age. However, this did not attain statistical significance. With regards to cost, it was found that the total number of extrapolated omeprazole 20 mg capsules utilized during the 6 months period for the study cohort was 30,812 capsules with a total cost of 863 R.O. Over prescriptions accounted for approximately 3620 extra capsules of omeprazole in 6 months with an additional cost of 101 R.O.

Conclusions: Generally, omeprazole prescribing at SQUH was appropriate in terms of indications but inappropriate in terms of length of therapy. Rational drug prescribing is needed to avoid unnecessary drug cost in order to maximize benefit from the available limited resources.

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PHYSICIANS’ REPORTED NEEDS OF DRUG INFORMATION AT POINT OF CARE IN SWEDEN
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Introduction: Relevant and easily accessible drug information at point-of-care is essential for physicians’ decision making when prescribing. The Summary of Product Characteristics (SmPC) is statutory information about drugs that is approved and issued by regulatory agencies. The current structure and format of SmPCs make it difficult to incorporate it into Decision Support Systems in association with relevant patient information from the Electronic Health Record (EHR). The aim of this study was to evaluate the perceived needs for drug information among physicians in Sweden.

Methods: We recruited three focus groups discussions with 18 physicians covering different specialities combined with a questionnaire administered at the beginning of the group discussions.

Results: Physicians highlighted their need for more drug information and requirement that it was integrated with user friendly features within the EHR. All physicians wished to have functions for automatically generated alerts for severe drug-drug interactions and adverse effects, and for calculating glomerular filtration rate to enable appropriate dose adjustments to be made for elderly patients and those with impaired renal function. Physicians also expressed a wish for visualisation of time schedules for drug therapy. Finally features making the electronic communication with colleagues possible and the drug information more searchable were also reported.

Conclusions: Most of the required information from physicians seems to be possible to transfer from the SmPCs to decision support systems (DSS). These results are also a useful step to further investigate the physicians’ needs in term of content, and the restructuring needs for compatibility with DSS functionalities, at European level.

P247
CONSUMPTION TREND ANTIBIOTICS IN CLINICAL CENTER OF THE SARAJEVO UNIVERSITY
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Antibiotics are substances produced by various species of microorganisms (bacteria, fungi, actinomycetes) that suppress the growth of other microorganisms and eventually may destroy them. However, common usage often extends the term antibiotics to include synthetic antibacterial agents, such as the sulphonamides and quinolones, which are not products of microbes. The goal in this study is establishing consumption a certain group of antibiotics in Clinical pharmacy in Clinical Center of the Sarajevo University. The method as to collect the data of consumption of antibiotics in Clinical pharmacy. The most trend consumption of antibiotics is registered ceftriaxone (amp. a 1000 mg) R² = 0.97, cefazidine (amp. 500 mg) R² = 0.854, and the most negative trend registered by ceftiraxone (amp. 1500 mg) R² = 0.031. To conclude the antibiotics have generally positive trend in investigation period, except in 2007 year were registered negative trend.

P248
DESCRIPTION OF THE WHO CORE DRUG USE INDICATORS AT KUWAIT AND SUDAN
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Introduction: Drug use studies are important tools to assess whether medicines are rationally used and also to develop comprehensive drug policy for better health care delivery. Indeed in Kuwait, information on drug use at various levels of the health care system is lacking. Also previous studies in Sudan have reported drug use patterns at primary care facilities and hospitals; whilst information of drug use practices in the paediatric population is lacking. These studies were designed to investigate current prescribing and dispensing practices using WHO core drug use indicators in Kuwait and Sudan.
Methods: Descriptive, quantitative and cross-sectional studies were conducted in Kuwait and Sudan. In Kuwait, 50 primary health care centers were selected using stratified and systematic random sampling. In Khartoum State, the four major specialized teaching paediatric were included. In Kuwait, prescribing indicators were investigated through collection of data on 5000 prescriptions of all age groups, determination of consultation and dispensing times for 2500 patients, and through the interview of 1500 patients for the evaluation of dispensing practices (January to October 2009). In Khartoum State, prescribing was assessed through a collection of 600 prescriptions, determination of consultation and dispensing times for 600 patients, and through the interview of 600 parents for the evaluation of dispensing practices (July to October 2008). In both studies, data were collected prospectively using systematic random sampling.

Results: In Kuwait, the mean number of drugs prescribed per prescription, 2.9; percentage prescribed by generic name, 17.7%; percentage of prescriptions involving an antibiotic, 39.1%; percentage of prescriptions with an injection prescribed, 9.1%. The mean consultation and dispensing times were 2.8 min and 54.6 s, respectively. The percentages of drugs actually dispensed, 97.9%; drugs adequately labelled 66.9%, whilst 26.9% of patients demonstrated adequate knowledge of all drugs dispensed for them. In Khartoum State, the mean number of drugs prescribed per prescription, 2.0; percentage prescribed by generic name, 49.3%; percentage of prescriptions involving an antibiotic, 81.3%; percentage of prescriptions with an injection prescribed, 3.5%. The mean consultation and dispensing times were 4.7 min and 28.2 s, respectively. The percentages of drugs actually dispensed was 80.1%; drugs adequately labelled 55.7%, whilst 83.5% of parents knew the correct dosage of all drugs dispensed, 97.9%. In Khartoum State, the mean number of drugs prescribed per prescription, 2.0; percentage prescribed by generic name, 49.3%; percentage of prescriptions involving an antibiotic, 81.3%; percentage of prescriptions with an injection prescribed, 3.5%. The mean consultation and dispensing times were 4.7 min and 28.2 s, respectively. The percentages of drugs actually dispensed was 80.1%; drugs adequately labelled 55.7%, whilst 83.5% of parents knew the correct dosage of all drugs dispensed, 97.9%. In Khartoum State, the mean number of drugs prescribed per prescription, 2.0; percentage prescribed by generic name, 49.3%; percentage of prescriptions involving an antibiotic, 81.3%; percentage of prescriptions with an injection prescribed, 3.5%. The mean consultation and dispensing times were 4.7 min and 28.2 s, respectively. The percentages of drugs actually dispensed was 80.1%; drugs adequately labelled 55.7%, whilst 83.5% of parents knew the correct dosage of all drugs dispensed, 97.9%. In Khartoum State, the mean number of drugs prescribed per prescription, 2.0; percentage prescribed by generic name, 49.3%; percentage of prescriptions involving an antibiotic, 81.3%; percentage of prescriptions with an injection prescribed, 3.5%. The mean consultation and dispensing times were 4.7 min and 28.2 s, respectively.

Conclusions: The findings of both studies indicate problem areas in prescribing and dispensing practices. Cost-effective, multifaceted interventions to improve current practices are needed.

P249 CHRONIC BENZODIAZEPINE USE IN NURSING HOME RESIDENTS: A CLOSER LOOK INTO INDICATIONS AND DOSAGES.

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Introduction: International guidelines discourage the chronic use of benzodiazepines and related Z drugs (BZD/Z). Reliable pharmaco-epidemiological data on prevalence, split per indication and supplemented with indication-specific dosage information is lacking for the elderly in different health settings, including nursing homes.

Methods: The medication charts from a representative sample of 1730 residents of 76 nursing homes in Belgium (the 2006 PHEBE Study) were analyzed for prevalence of use of BZD/Z, indication and dosages. The ATC classification was used to differentiate BZD/Z as hypnotics, anxiolytics, and Z-drugs. Dosages were expressed in Defined Daily Doses (DDD).

Results: Chronic use of BZD/Z was observed in 50.1% of the residents, with multiple use in 7.6%. Among the chronic users, using only one BZD/Z, the leading indication was insomnia (59%) followed by anxiety (17%) and sedation (10%), multiple indications (10%), and ‘other indications’ (6%). For insomnia, physicians prescribed mostly (46%) hypnotics (N05CD), but also anxiolytics (N05BA) and Z drugs (N05CF), each in 27% of the cases. For anxiety, physicians prescribed predominantly anxiolytics (96.8%) and only 2 hypnotics (lorazepam and triazolam). Average dosage was 1 DDD for most drugs. Daily dosages exceeding 2 DDD occurred in 0.2% for the indication insomnia and in 2.2% for anxiety.

Conclusion: Insomnia is the predominant indication of BZD/Z use in nursing homes. The average dose used in this indication is 1 DDD, which is double the recommended dose for elderly. The use of excessive doses in anxiety and sedation is limited to a minority of patients.

P250 COMPARISONS OF OUTCOMES BETWEEN NEW USERS OF ROSIGLITAZONE AND PIOGLITAZONE IN AUSTRIA

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Introduction: Meta-analyses of randomized trials have shown that, compared to control treatment, patients using rosiglitazone experienced higher rates heart failure and myocardial infarction. Patients using pioglitazone had an increased risk of heart failure but a lower incidence of a composite cardiovascular endpoint including myocardial infarction, stroke, or death. Observational studies found higher rates of cardiovascular events in patients receiving rosiglitazone vs. pioglitazone. In the fall of 2010, rosiglitazone was removed from the European market and the United States Food and Drug Administration (FDA) drastically limited its use. The present study was undertaken to compare incidence rates of cardiovascular outcomes (congestive heart failure, myocardial infarction and stroke) for diabetes patients initiating rosiglitazone vs. pioglitazone treatment.

Methods: All patients insured through the nine Provincial Sickness Funds as well as the Insurance Funds for Farmers (SVB) and the Self-Employed (SVA), January 2006 to June 2010. We used billing claims for all submitted health-care encounters and filled prescriptions as well as enrolment files for all patients who filled at least one prescription for any oral hypoglycemic medication during the study period. Patients were categorized as new pioglitazone or rosiglitazone users based on first prescriptions for these drugs (single-agent or combination products, TZD’s) filled after >6 months of claims available without such a specific medication claim. Patients were then followed until a study outcome occurred or censored at the date of last service after 3 months of complete inactivity regarding any billed health care services or crossover between the two study medications. Statistical analyses were performed using univariable and multivariable Cox proportional hazards regression.

Results and Conclusions: Of the 30,177 total new users of TZD’s, 69.4% used pioglitazone and 30.6% used rosiglitazone. Preliminary analyses indicate that little channeling of the two study drugs occurred in Austria during the study period. If anything, initiators of pioglitazone appeared to be slightly older. Patients in both groups were similar with regard to sex, concomitant medication use and waiver of co-payment (a proxy for socioeconomic status). The multivariable analyses are compatible with an increased risk of myocardial infarction (Hazards ratio: 0.64; 95% Confidence Interval: 0.47–0.87) among rosiglitazone users (vs. pioglitazone users) with no association between TZD choice and the other outcomes, hospitalization for heart failure or ischemic stroke.

P251 PHARMACOLOGICAL MANAGEMENT OF OBESITY IN THE NATIONAL HEALTH AND NUTRITION EXAMINATION SURVEY (NHANES) 2007–2008

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Introduction: Obesity is a growing problem worldwide. We set out to investigate the use of anti-obesity drugs in the United States in recent years.

Methods: We included 2630 men and 2702 women who took part in NHANES in 2007–2008. We analyzed their demographic and anthropometric data, and their weight and drug history. Sampling weights were used to adjust for non-response bias and the oversampling of blacks, Mexican Americans, and the elderly.
Results: 45.9% of men and 45.0% of women were candidates for anti-obesity medication (initial body mass index $\geq 30$ kg/m$^2$, or $\geq 22$ kg/m$^2$ in the presence of other risk factors [e.g. hypertension, diabetes or dyslipidemia]). Among these participants, 85.1% considered themselves overweight and 90.1% would like to lose weight. However, only 61.9% had dietary changes, 36.5% exercised, 3.7% took non-prescription diet pills and 2.2% took prescription diet pills to control weight during the preceding year. During the preceding month, 0.5% and 0.1% of participants were taking phentermine and orlistat respectively. There were no participants on sibutramine.

Conclusions: Obesity is highly prevalent in the United States, but only a very small percentage is on anti-obesity medication. The withdrawal of sibutramine in October 2010 would have minimal impact on the general population. While improvements in pharmacological treatment of obesity are needed, our study revealed that there is also a need for more lifestyle changes in the majority of obese individuals.

P252
RISK OF LIVER INJURY IN PAEDIATRIC POPULATION: DATA MINING ON ELECTRONIC HEALTHCARE DATABASES IN EUROPE
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Introduction: Drugs are one of most important causes of liver injury in paediatrics but so far, the incidence rate of drug-related hepatic injury has only been estimated in the general population. A retrospective popu-
lation-based cohort study was performed to estimate the incidence rates of hepatic injury in children and to identify the drugs associated with hepatic injury.

Methods: Between 1 January 2001 and 31 December 2008 data on population aged 1–18 years were collected using three general practice databases in two European countries: in The Netherlands, the Integrated Primary Care Information (IPCI) database, and in Italy, Pedianet (cover-
ing children up to 14), and Health Search/Thales (HSD) (covering children from 14 years). Potential cases of hepatic injury were identified through codes, free text and lab values search. All potential cases were manually validated using specific algorithms by two assessors, blinded to drug exposure. Crude incidence rates (IR; 95% CI) of hepatic injury by country and age and gender-adjusted relative risks (RR; 95% CI) were estimated using Jerboa (http://www.euadr-project.org/).

Results: In the study cohort of 319,755 subjects aged 1–18 years, contributing 1,149,066.72 person-years (PY) of follow-up, we identified 871 new definite cases of hepatic injury (46 cases in IPCI, 825 cases in HSD and Pedianet). The incidence rate for hepatic injury was 92.6 (95% CI 86.5–99.2) per 100,000 PY in Italy and 17.9 (13.3–23.6) per 100,000 PY in the Netherlands. Pooled data showed a statistically significant age and gender adjusted increased risk of hepatic injury for antibacterials (RR 13.4; 95% CI 10.9–16.4), followed by corticosteroids for systemic use (11.7;6.3–21.9), drugs for functional gastrointestinal disorders (9.7;3.6–
26.1), NSAIDs (7.6;4.0–14.8), agents for acid related disorders (6.4; 2.7–
15.5), antiepileptics (5.5;3.2–9.5) and drugs for obstructive airway dis-
dises (2.6;1.7–4.1).

Conclusions: The incidence of hepatic injury is rare in the paediatric population and heterogeneous between the two European countries. Corticosteroids, antibacterials, NSAIDs, antiepileptics and drugs for obstructive airway diseases are associated with an increased risk of hepatic injury.

P253
EVALUATION OF CARDIOVASCULAR RISK WITH NSAIDS: LIMITATIONS ARISING FROM INCONSISTENT REPORTING IN PHARMACOEPIEMIDIOLOGICAL STUDY
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Concerns about cardiovascular risks with NSAIDs arose from random-
ized studies of gastro-intestinal safety, Alzheimer’s disease and tumor protection. Subsequent pharmacoepidemiological studies explored risks associated with NSAID use in general populations. Factors important in judging risk include: dose, underlying cardiovascular risk, timeframe for risk onset. We investigated how these factors were reported in the phar-
macoepidemiological studies.

Methods: Review of studies included in a systematic review and meta-
analysis of cardiovascular risk.

Results: Of 51 eligible studies, 28 case-control studies included 184,946 cardiovascular events, 23 cohort studies described outcomes in $\geq$2.3 million exposed individuals.

Dose: Of 38 studies reporting on rofecoxib and/or celecoxib, 14 and 8 respectively reported risk with different doses; all used the same low/ high dose stratifications. Of 42 studies reporting on one or more of three commonly-used NSAIDs (ibuprofen, diclofenac, naproxen), only nine reported low/high dose risk estimates and three different dose stratifica-
tions were used.

Background: cardiovascular risk: Fourteen studies provided information on ‘high’ and ‘low’ risk populations. ‘High’ and ‘low’ risk definitions varied between studies.

Timeframe for risk onset: Thirty studies, reported events among new-
users of NSAIDs; nine found risk elevated within 30 days; three within 14 days.

Conclusion: In assessing NSAID-associated cardiovascular risk, a minority of studies reported three factors that are important potential effect modifiers. Among studies providing this information, between-
study variability in defining factor-related parameters impeded assess-
ment of variation in cardiovascular risk. Agreement on reporting criteria would greatly improve the value of data from pharmacoepidemiological studies of NSAID-related risk and permit greater precision in risk esti-
mates.

P254
DRUG-RELATED PROBLEMS IN PAEDIATRIC PATIENTS IN UK: WHAT IS THE EXTENT OF HOSPITAL PHARMACIST’S CONTRIBUTION?
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Introduction: Limited research has investigated the incidence and nature of drug-related problems (DRPs) in children. The size of DRPs in paediatric population in UK is unknown. Our aim is to identify the inci-
dence of DRPs in children admitted to paediatric wards or attending Accident & Emergency (A&E) department in a UK hospital and to eval-
uate pharmacist contribution in solving a problem.

Methods: Prospective cohort study was conducted in a paediatric hospita-
lar and included children aged 0–18 years admitted to medical ward, pa-
ediatric intensive care unit (PICU) and neonatal intensive care unit (NICU) or attending the A&E department during a 3-month study period. DRPs were classified based on PCNE classification system (V5.01). Pharmacist intervention and time spent per DRP cases were recorded.

Results: Four hundred and eighty-three children were included from the three wards and one A&E department. Mean age of the study population was 4.5 years ($\pm$4.9), 60% were male. 161 of the 483 patients suffered from 237 DRPs. Overall DRPs incidence in the study cohort was 33.3% (95% CI, 29.1–37.7). Incidence was found to be highest in PICU (60.0%; 95% CI, 45.2–73.6). 64.1% of DRP cases were preventable and out of these 82.9% were intervened by pharmacist. Overall average time
needed by pharmacist for intervention and solving a DRP was 9.8 min (±8.8).

**Conclusion:** This study showed that DRPs are a significant problem in paediatric population in UK. Also, hospital pharmacist’s has a significant role on improving the quality of paediatric patient care as well as the economic impact on healthcare system.

**Funding:** ManMed Award 2009 from NPPG.

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**P255**

**HORMONE REPLACEMENT THERAPY IN SOUTH AFRICA AND THE IMPACT OF THE WOMEN’S HEALTH INITIATIVE STUDY**

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A study to determine the impact of the Women’s Health Initiative (WHI) study on the prescribing of hormone replacement therapy (HRT) from 2002 to 2005 in South Africa found that the discontinuation rate for HRT following publication of the WHI study increased from 3.47% to 7.54%, while the initiation rate decreased from 15.34% to 9.10%. The primary aim of this follow-up study was to determine the prescribing patterns of HRT to establish if major changes have taken place since the last study. A retrospective, cross-sectional drug utilisation study was conducted. Prescription data of a medical aid administrator were retrospectively analysed for 2008 and 2009. A total of 33556 HRT products were prescribed to 3665 patients over the 2 years. The average age of patients was 50.78 (SD = 12.51) years in 2008 and 50.21 (SD = 12.41) years in 2009. Patients received an average of 5.80 (SD = 4.09) products during 2008 and 6.50 (SD = 4.85) products during 2009. Oestrogens (ATC code G03C, excluding tibolone) were the most frequently prescribed (71.83% in 2008/2009 compared to 72.28% in 2005), followed by progestogens and oestrogens in combination (20.09% in 2008/2009 and 20.39% in 2005). Progestogens accounted for 3.49% of prescribing frequency in 2008/2009 compared to 2.20% in 2005. No major differences in the prescribing of HRT since 2005 could therefore be detected. Adverse effects linked to HRT use may be affected by time after onset of menopause when HRT was started. A longitudinal study over several years which include diagnoses is recommended to be conducted in South Africa.

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**P256**

**RATIO ‘INHALED CORTICOSTEROIDS TO TOTAL ANTI-ASTHMA DRUGS’, AND ASThma-RELATED HOSPITAL ADMISSIONS**

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**Financial support:** This study was supported by the French National Health Insurance Agency.

**Background:** The inadequate use of inhaled corticosteroids (ICS) remains an issue in asthma. The ratio of ICS units to total anti-asthma medications dispensed (ICS/R03 ratio) may be useful to assess the risk of severe exacerbations, leading to asthma-related hospitalisations (ARHs). We tested this hypothesis using claims data.

**Objectives:** To verify in claims databases whether patients with a higher ICS/R03 ratio experience fewer ARHs.

**Methods:** A random sample of patients aged 16–40, with regular use of respiratory drugs in 2005 was selected from the French national claims database (EGB). Three groups were defined according to the value of ICS/R03 ratio in 2007: 0% (‘non users’), <50% (‘inadequate users’) and ≥50% (‘adequate users’). ARHs in 2007 and 2008 were compared between the three groups.

**Results:** Among 1812 patients (mean age = 32 year-old, 54.2% females), non users, inadequate users and adequate users were 17%, 37% and 46%, respectively. ARH rate was 0.9% in 2007 (0.7% in 2008). Patients with ARH in 2007 were more numerous (P = 0.0006) among inadequate users (2.10%), compared with adequate users (0.24%) and non users (0.32%). Differences were also observed in mean ARH-induced costs: 2.16 €, 48.30 € and 3.70 € for non users, inadequate, and adequate users (P = 0.0005). Conversely, no conclusive results were obtained with the number of quarters containing ≥ one dispensing of ICS, nor with the proportion of days covered by ICS in 2007.

**Conclusions:** Patients with ICS/R03 ratio ≥50% experience fewer ARHs than inadequate users, suggesting improved asthma control. ICS/ R03 ratio may help identify asthma patients at risk of ARHs in administrative databases. The differences observed between different levels of exposure will be discussed.

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**P257**

**STATINS AND NEW-ONSET DIABETES IN THE PRIMARY CARE POPULATION**

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**Introduction:** Recent meta-analyses of randomized controlled trials have shown increased risk of new-onset diabetes with statins [1, 2]. The risk of new-onset diabetes with statins in the general primary care population needs to be determined.

**Methods:** A retrospective cohort study was performed using the Irish Health Services Executive-Primary Care Reimbursement Services national pharmacy claims database. Individuals prescribed any medicines were identified from January 2001 to January 2009 (n = 1,233,166). Patients newly prescribed statins from 1st January 2002 to 31st December 2007 were identified from the cohort (n = 197,138). Patients with new-onset type 2 diabetes were identified as individuals newly initiating oral antidiabetic therapies (n = 25,820). Those prescribed statins in the same month or after initiation of antidiabetic therapies were excluded. Adjusted hazards ratios (HR) with 95% confidence intervals were calculated to examine the association between prescribed statins and time to new-onset diabetes using Cox proportional hazard regression compared to those prescribed none. A test for linear trend was used to examine the dose and duration response relationship of statins on new onset diabetes.

**Results:** Any statin was associated with increased risk of new-onset diabetes (HR 1.18[1.15, 1.22]) in this cohort. Increased risk of new-onset diabetes was found with monotherapy of rosuvastatin (HR 1.41[1.31, 1.52]), atorvastatin (HR 1.23[1.19, 1.27]) and simvastatin (HR 1.15[1.05, 1.25]). A significant linear trend association between duration and dose of treatment and new-onset of diabetes was observed with all classes of statins (P < 0.0001).

**Conclusion:** An increased risk of new-onset diabetes was found in those prescribed statins showing linear association with duration and dose of treatment.

**References:**


P258
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Introduction: Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most frequently used medications worldwide and as such are frequently used to treat common musculoskeletal conditions. The widespread use of NSAIDs has led to increased prevalence of adverse effects of these drugs. For these reasons it is important to analyze the amount and pattern of use of NSAIDs in Serbia and to compare these parameters with those in other countries.

Methods: The analysis covered the use of NSAIDs in Serbia and Hungary during the 4-year period 2005–2008. Data on consumption of drugs of M01A group [according to the Anatomical Therapeutic Chemical classification (ATC)] were obtained from the annual reports of the Agency for Drugs and Medical Devices of Serbia and from results published in original report on trends in NISIA market (Inotai et al. Pharmacoeconomics and Outcomes Research 2010; 19: 183–190) Results were expressed as the number of defined daily doses/1000 inhabitants/day.

Results: In Serbia, use of diclofenac comprised 50% of total NSAIDs consumption (without a tendency of decrease in its consumption), followed by ibuprofen (without a tendency of increase in its prescription), while in Hungary diclofenac use was decreasing over the years and ibuprofen consumption has increased nine times.

Conclusions: Considerable improvement has been made in Hungary, concerning pharmaceutical practice. Improvement of the quality of prescription and use of NSAIDs in the population in Serbia can be achieved by continuous education of physicians and by informing general population about the risks of inappropriate drug use.

Key words: ATC/DDD methodology, non-steroidal anti-inflammatory drugs, pharmacopeidemiology, Serbia, Hungary.

Acknowledgments: This research is part of the project No. 41012 which is financially supported by the Ministry of Science of the Republic of Serbia.

P259
ASSESSMENT OF CONTROLLED MEDICINES PRESCRIPTION PATTERNS IN THE CONTEXT OF GREEN AND RED COLOURED SCRIPTS
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Introduction: In Turkey, controlled ‘psychotropic drugs’ and ‘narcotic drugs’ are allowed to use in the therapy under the condition if they are written out on ‘green coloured scripts (GCS)’ and ‘red coloured scripts (RCS)’ respectively. In this study, it was aimed to reveal controlled medicines (CM) utilization by comparing content of GCS and RCS.

Method: A total of 52000 GCS and RCS (4000 prescriptions/month) were collected retrospectively (December 2008-December 2009) from Provincial Health Directorate of Istanbul. GCS and RCS were analyzed by patients’ gender, indications, physicians’ specializations and CM-related details.

Results: The majority of scripts were green coloured (78.6%). GCS were mostly prescribed by physicians from psychiatry (37.4%) while RCS were mostly prescribed by physicians from child psychiatry (35.0%). The most frequently prescribed CM on GCS was alprazolam (39.4%) and on RCS was methylfenidate (51.1%). The most commonly written indication on GCS was anxiety (30.8%) and on RCS was ‘attention deficiency-hyperactivity disorders’ (53.5%). GCS was prescribed mostly for women (55.4%) whereas RCS was prescribed mostly for men (67.8%) (P < 0.001). Prescription content was found to be similar for each month.

Conclusion: The findings indicated that there was a tendency towards use of controlled psychotropic medicines mostly by women while narcotics mostly by men. Frequently prescribed CM were prescribed by the psychiatrists. There was no seasonal difference among the prescribed CM in each month. All these findings may serve as a guide for the development of CM follow-up systems and regulations.

P260
ANTIPSYCHOTIC DRUG USE IN NURSING HOME RESIDENTS WITH PARKINSON’S DISEASE
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Introduction: Guidelines caution against chronic use of antipsychotics, typical antipsychotics in particular, for older adults with Parkinson’s disease (PD). Our study goal was to determine the prevalence of antipsychotic utilisation in Belgian nursing home residents with PD, with attention to drug choice and resident characteristics.

Methods: Prescribing in Homes for the Elderly in Belgium (PHEBE, 2006) was a cross-sectional study conducted in 76 nursing homes investigating drug utilisation in general. Antipsychotic drugs were categorised into typical and atypical with the ATC classification.

Results: Parkinson’s disease was diagnosed by the GP in 8.3% (n = 144) of the residents (n = 1730). Among residents with PD, mean age (84 years range 56–101), prevalence of dementia (46%), the number of different drugs chronically used (eight drugs range 1–17), the prevalence of antipsychotic use (n = 41, 29%), and the proportion of typical (36%, mainly haloperidol) and atypical agents (64%, mainly risperidone) did not differ significantly from the nursing home population. In residents with PD using antipsychotics, insomnia was present in 65% and dementia in 62% vs. resp. 41% and 42% among non-users (resp. P = 0.009 and P = 0.026).

Conclusion: Prevalence and drug choice of antipsychotics was not different among nursing home residents with and without PD, indicating that GPs did not take PD into account as a risk factor. Drug Utilisation Review efforts are needed to reduce the high prevalence and the proportion of typical antipsychotic users in older adults with PD.

P261
USE OF ANTIDEPRESSANTS IN BELGIAN NURSING HOMES
Bourgeois J.

Introduction: Reliable pharmaco-epidemiological data on prevalence, split per indication and supplemented with indication-specific dosage information about antidepressants (AD) is lacking for the different health settings, including nursing homes.

Methods: The medication charts from a representative sample of 1730 residents of 76 nursing homes in Belgium (PHEBE Study) were analyzed. The ATC classification was used to differentiate the AD’s. Dosages were expressed in Defined Daily Doses (DDD).

Results: Chronic AD use was present in 40.4% of the Belgian nursing home residents, with multiple AD use in 7.0%. A Selective Serotonin Reuptake Inhibitor (SSRI) was prescribed in 63% of the users (N = 695), trazodone in 27%, a Selective Serotonin and Noradrenalin Reuptake Inhibitor (i.e. venlafaxine) in 10%, and tricyclic antidepressants (TCAs) in 9%. The physician labeled 35.7% of the residents as depressed. Of these patients 80% were treated with an AD (predominantly SSRI for the indication of ‘depression’).

Among the 10.0% patients treated with an AD and not labeled as depressed (n = 175), 4/10 had ‘depression’ as indication on the prescription (SSRI), 4/10 ‘insomnia’ (Trazodone), 1/10 ‘multiple indications’, and 1/20 ‘neuropathic pain’ (TCA). Prescribed daily dose was mostly 1 DDD or less, and rarely exceeded 2 DDD. There was a decrease of antidepressant use in residents with deteriorating dementia.

Conclusion: Belgian nursing homes residents, labeled as depressed, are appropriately treated with SSRI. Inappropriate use of TCA is still present. Further research will determine whether the low dosages and the lower prevalence of AD in progressing dementia indicates underuse of AD in nursing homes.
P262
PREGNANCY OUTCOME FOLLOWING MATERNAL EXPOSURE TO STATINS: A MULTICENTER PROSPECTIVE STUDY
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Introduction: Statin use for the treatment of hypercholesterolemia in women of childbearing age is increasingly common. However, published data on pregnancy outcome after exposure to statins are scarce and conflicting. This contribution addresses the safety of exposure to statins during pregnancy.

Method: In a multi-center (n = 11) observational, prospective study we compared the outcomes of 249 women exposed during the 1st trimester of pregnancy to simvastatin (n = 124), atorvastatin (n = 67), pravastatin (n = 32), rosuvastatin (n = 18), fluvastatin (n = 7) or cerivastatin (n = 1) with a control group exposed to agents known to be non-teratogenic (n = 249). The data were collected by members of the European Network of Teratology Information Services (ENTIS) during individual risk counseling between 1990 and 2009. Standardized procedures for data collection were used in each center.

Results: The difference in the rate of major birth defects between the statin-exposed group and the control group was not statistically significant (4.0% vs. 2.7% OR 1.5; 95% CI 0.5–4.5, P = 0.44). The crude rate of spontaneous abortions (12.8% vs. 7.1%, OR 1.9, 95% CI 1.0–3.6, P = 0.04) was higher in the exposed group. However, after adjustment to maternal age and gestational age at initial contact, the difference became statistically insignificant. The rate of elective pregnancy-termination (8.8% vs. 4.4%, P = 0.05) was higher and the rate of deliveries resulting in live births was significantly lower in the statin exposed group (77.9% vs. 88.4%, P = 0.002). Prematurity was more frequent in exposed pregnancies (16.1% vs. 8.5%; OR 2.1, 95% CI 1.1–3.8, P = 0.02). Nonetheless, gestational age at birth (median 39 weeks, IQR 37–40 vs. 39 weeks, IQR 38–40, P = 0.27) and birth weight (median 3200 g, IQR 2835–3590 vs. 3250 g, IQR 2880–3600, P = 0.95) did not differ between exposed and non-exposed pregnancies.

Conclusion: This study did not detect a clear teratogenic effect of statins. Its statistical power however is not sufficient to reverse the recommendation of treatment discontinuation during pregnancy. At most, the results are reassuring in case of inadvertent exposure.

P264
SELECTIVE SEROTONIN REUPTAKE INHIBITOR AND HEMOSTASIS DISORDERS IN SURGERY
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Introduction: The use of selective serotonin reuptake inhibitor (SSRI) has been associated with an increased bleeding tendency. An increased volume of blood loss was found during orthopedic surgery in patients using SSRIs. However, preoperative use of SSRIs was associated with need blood transfusion only in one study. These results are contradictory about the impact of a possible impaired hemostasis associated with the perioperative use of SSRIs.

Patients and Methods: A retrospective cohort study was conducted among the patients who underwent elective surgery in ‘Cova da Beira’ Hospital Center (CBHC) during 1 year. The patients that take were taking anti-inflammatory non steroid or antithrombotic agents were excluded. Two groups were evaluated, CCB users and nonusers of chronic medication.

Results: The study included 295 patients, with a mean age of 48.6 years and 51.9% were men. There was 267 nonusers of chronic medication, 28 CCBs users. Three CCBs users (10.7%) and 20 nonusers of chronic medication (7.5%) had the end point. There was no significant association between the use of CCB and the occurrence of the end point (P = 0.481).

Conclusions: The incidence of haemorrhage or the need of transfusion in perioperative period was not associated with the chronic use of CCBs.

P265
CONSUMPTION OF DRUGS ACTING ON CARDIOVASCULAR SYSTEM IN SERBIA
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Serbia is the 4th country in the world regarding the number of citizens aged over 65 and in 2007. Had 3rd mortality rate due to cardiovascular diseases in the world. One of the consequences of such demographic occurrences is the increase of the use of cardiovascular drugs.
The aim of this study is to analyse consumption of cardiovascular drugs in Serbia. This is a retrospective study. The data on drug utilization from 2004 to 2007 were obtained from the reports of the Medicines and Medical Devices Agency of Serbia. The ATC/DDD methodology was used. The results were expressed as a number of DDD/1000 inhabitants/day (DDD/TID).

Cardiovascular drugs (ATC group C) dominated in total drugs consumption (35.25%). Over the study period consumption of C drugs increased significantly ($r = 0.93$, $P < 0.05$). ACE inhibitors were dominant in total C drugs use with 41% on average. Beta blockers and calcium channel blockers participated in C drugs use equally, 11% each. The most striking is the increase of the use of statins, which in 2004 and 2005 did not participate in the consumption of C drugs, but from 2006. Simvastatin became one of the 10 most frequently prescribed. The increase in total C drugs consumption in Serbia was mainly on the account on ACE inhibitors and the utilization of these agents also increased significantly ($r = 0.92$, $P < 0.05$).

This study reveals the increasing trend towards consumption of C drugs in an ageing Serbian population and corroborates the alarming epidemiologic data on the incidence on cardiovascular diseases.

P266
UTILIZATION OF ROSIGLITAZONE BETWEEN 2001 AND 2010 IN HUNGARY
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Introduction: Rosiglitazone (Avandia) became available for the treatment of type 2 diabetes in Hungary in 2001, and its combination with metformin (Avandamet) was introduced in 2005, and the combination with glimepiride (Avaglim) in 2007. Due to concerns on the cardiovascular safety of rosiglitazone containing medication the European Medicines Agency recommended the suspension of marketing authorization of these medication on 23rd September 2010. Doctors were recommended to stop prescribing these medication, but pharmacies could dispense them on previously issued prescriptions. These medication were withdrawn on 15th February 2011.

The aim of this study was to analyze the changes in the utilization of rosiglitazone during its availability in Hungary between 2001 and 2010.

Methods: Crude national drug utilization data was obtained from IMS PharmMIS Consulting Company regarding the years between 2001 and 2004, and from the administrative drug dispensing database of the Hungarian National Health Fund Administration regarding the years between 2005 and 2010. Data were analyzed according to the ATC/DDD (WHO) methodology. Data were expressed as defined daily doses per 1000 inhabitants per day (DDD/TID).

Results: Between 2001 and 2004 the utilization of rosiglitazone was negligible, reaching 0.079 DDD/TID in 2004, which was only 0.19% of the total oral antidiabetic drug use. Popularity started to rise with the introduction of the combination of rosiglitazone with metformin (Avandamet) in 2005. Its utilization peaked in 2008, when Avandamet use was 1.875 DDD/TID, and the total rosiglitazone use was 2.046 DDD/TID, which was 4.59% of the total oral antidiabetic drug use. The use of rosiglitazone and glimepiride combination remained marginal, peaking in 2009 at 0.051 DDD/TID. In September 2010, total rosiglitazone use was 1.580 DDD/TID, which dropped to 0.850 DDD/TID in October, and in December it was only 0.137 DDD/TID.

Conclusions: Rosiglitazone had a limited use throughout its one decade of history. As a limited number of patients had used rosiglitazone, it is unlikely to have caused a large number of excess cardiovascular events in Hungary. Its withdrawal does not cause disruption or any essential problem in the treatment and care of patients with type 2 diabetes.

P268
PATTERN OF MEDICATION USE IN ELDERLY INSTITUTIONALIZED
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Introduction: Progressive aging of the population is a reality as the fact that lead to the simultaneous existence of multiple diseases, particularly chronic, in the same person. Being the age group that consumes more drugs, the elderly, are highly susceptible to drug interactions and adverse drug reactions. The aim was to analyze the pattern of drug consumption in elderly.

Methods: Were described the most consumed pharmacotherapeutic groups, calculated the proportion of elderly chronically medicated, average number of medications consumed (not chronic/chronic use or SOS) and also checked the influence of gender and age. The study was performed in a nursing home (center of Portugal), and consisted in a correlational cross-sectional descriptive study.

Data were collected from the therapeutic files. Results were analyzed in SPSS v.16.

Results: The sample consists of 71 elderly, 49 (69%) were female and 38 (53%) aged 65 or more years. The elderly consume in SOS at least one medication/day and a maximum of 2.13 medicines in acute situations and 12 in chronic use. Average is 6.65 drugs/day, 1.18 in SOS and 5.34 in chronic use. Women’s are the major consumers of drugs either in SOS or chronic use or even in acute situations.

Conclusion: The pharmacotherapeutic groups most consumed by the elderly were ‘Anti-hypertensive’s’ (74.65%) and ‘Psychotropics’ (70.42%). The results of this study show a high pattern of drug use among persons aged 65 years or more and data show us that system needs to be changed to get a more efficiently survey in prescriptions.
**P269**

**DIAGNOSTIC AND TREATMENT PATTERNS FOR ACUTE MALE URETHRITIS IN 4 RUSSIAN CITIES**


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**Objectives:** Making an etiological diagnosis of acute male urethritis poses a significant challenge because of the lack of reliable diagnostics methods (DM) for identification of atypical pathogens in routine clinical practice in Russia. We aimed our study to determine the diagnostic and treatment patterns for acute male urethritis in Russia.

**Methods:** Retrospective cross-sectional study was conducted during 2009 in 5 centers in 4 cities of the Central Part of Russia (Kaluga, Pskov, Smolensk – 2 sites and Tula). Data on diagnostic and treatment approaches for acute urethritis in male subjects ≥16 years old were collected and analyzed with the use of specially designed case report forms.

**Results:** 556 cases of acute male urethritis were analyzed during study period: Smolensk #1 (120/21.6%), Pskov (150/27%), Kaluga (95/17.1%), Smolensk #2 (102/18.3%), Tula (89/16.0%). The average age of patients was 29 years (16–68 years). The following DM were used in 96.4% of cases (C. trachomatis: PCR (138/24.8%), ELISA (156/28.1%); N. gonorrhoeae and T. vaginalis, respectively: urethral smear microscopy (445/80.0%), PCR (109/19.6%) and 130/23.4%; U. urealyticum and M. hominis, respectively: bacteriology (140/25.2%) and 135/24.3%, PCR (135/24.3% and 133/23.9%); M. genitalium: PCR (135/24.3%). The most common diagnosis in Smolensk #1 was gonococcal urethritis (GU) (61.7%), in Pskov, Kaluga, Tula – non-gonococcal urethritis (NGU) (68.0, 54.7, 66.3%), in Smolensk #2 – unspecified urethritis (UU) (45.1%) and NGU (36.3%). Treatment patterns included 1) antimicrobials (ABx) solely – in 66.3%, and 2) ABx + non-ABx – in 32.2% of cases. The most often prescribed ABx were azithromycin (25.7%), ceftriaxone (17.4%), doxycycline (13.6%), metronidazole (11.4%), fluconazole (13.7%), josamycin (5.8) and ofloxacin (5.5%).

**Conclusions:** Use of PCR for atypical pathogens was rare (C. trachomatis 24.8%, U. urealyticum 24.3%, M. hominis 23.9%, M. genitalium 24.3%). Doubtful culture methods were used for detection of U. urealyticum and M. hominis (25.2% and 24.3%). ABx coverage for atypical pathogens was low in case of GU (<20% of total cases), GU + APV (<50%); TVU (<30%) and TVU + APV (<60%) that was not comply with modern practical guidelines of STD and acute urethritis therapy.

**P270**

**PHARMACIST–PATIENT COMMUNICATION MIGHT IMPROVE ADHERENCE TO MEDICATION BEHAVIOR: CROSS-SECTIONAL STUDY**

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**Introduction:** Patients’ adherence to chronic medication is poor. A survey in Zagreb from 2008 showed that adherence was only 41%, for which various reasons were found: personal behavior (forgetfulness, away from home), drug prescribing (dosage schemes, too many different drugs), doctor–patient and pharmacist–patient communication.

**Patients and Methods:** Patients with chronic diseases were interviewed during their visit to a public pharmacy (Pharmacia, Zagreb). A standard questionnaire to assess the adherence behavior rate was used along with the standard EQ-5D. A pharmacist–patient communication was evaluated using a set of eight specific questions. They were asked to visit the pharmacy within 30 days for the next prescribed packages of the same medication. The same interview was repeated.

**Results:** At the 1st visit, 152 patients accepted to take part in the survey. Among the prescribed medication, 52.8% was cardiovascular treatment. According to a self-reported interview, 65.1% of patients claimed to be adherent to medication. Only 87 patients (57.2%) showed up at the 2nd visit to the pharmacy and took part in the survey; 60 of them (69.0%) claimed to be adherent to medication. The evaluation of the pharmacist–patient communication showed significant improvement during the 2nd visit. Statistically significant was the answer to the procedure of loudly repeating the dosage scheme by patients (P < 0.05). The QoL parameters showed better results in chronic patients who were adherent to medication.

**Conclusion:** Patients’ adherence to medication behavior is a great challenge for efficient pharmacotherapy. The pharmacist–patient communication should be reassessed and improved.

**P271**

**REFILL-BASED COMPLIANCE RATES OF CALCIUM CHANNEL BLOCKERS USING RETROSPECTIVE MEDICINE CLAIMS DATA**

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**Objective:** To determine the refill-based adherence rates and to compare the costs associated with an under- or over-supply of calcium channel blockers (CCB).

**Research Methodology:** A non-experimental, quantitative retrospective drug utilization review was performed on medicine claims data from a pharmacy benefit management company in South Africa for the period January 1, 2005 until December 31, 2008. Refill-based adherence rates were calculated for 107,529 CCBs that were prescribed more than once during a 4-year period. The refill-based adherence rate was calculated per trade name by using the following equation: Refill-adherence rate = (total number of days of CCBs supplied – days supplied at the last refill)/(date last claimed – date first claimed). [RSA Rand (R)/US$ = 6.38112 (2005); 6.78812 (2006); 7.06926 (2007) and 8.27505 (2008)].

**Results:** Only 60.34% of CCBs had acceptable refill-adherence rates (between 90% and 110%). CCB with refill-adherence rates below 80% (possibly under-supplied) accounted for 35.65% of the CCB medicine items and represented 24% (N = R41,758,610.75) of the total cost (N = R174,105,357) of all CCBs included in the study. The average refill-adherence rates of male patients (91.46% SD = 149.93%) were higher than those for female patients (88.89% SD = 135.82%). The highest average refill-adherence rate was noted with CCBs containing amiodipine (93.39% SD = 143.4%). Only 54.4% of nifedipine containing CCBs had acceptable refill-adherence rates between 80% and 120%.

**Conclusion:** The financial implication of non-adherent use of CCBs is relatively large and efforts should be made to promote better adherence by patients.

**P272**

**THE INFLUENCE OF EDUCATION LEVEL ON SELF-MEDICATION AND MEDICINE CONSUMPTION AMONG PREGNANT WOMEN IN MONTENEGRO**

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**Introduction:** Self-medication (SM) is defined as treatment of common health problems with medicines especially designed and labeled for use without medical supervision and approved as safe and effective for such use. Anything a pregnant woman (PW) ingests or is exposed to could...
affect her fetus. The objective of this study was to evaluate SM among PW and its correlation with education level.

Methods: This cross-sectional pharmacoepidemiological study was conducted in Department of Obstetric and Gynecology of the General Hospital ‘Danilo I’ in Cetinje. A total of 246 PW were interviewed using a questionnaire over a period of seven consecutive weeks starting in October 2010, regarding socio-demographic data and pregnancy information, proportion of utilization of OTC medications, and prescription only medications (POM), supplement intake and utilization of herbal medication. According education level, PW were classified into undergraduate and graduate group. Data were analyzed using EPI INFO version 10 for Windows. Chi-square test was used to test the significance.

Results: Approximately 5% (n = 13) of PW reported a chronic disease. Mean medication intake per PW was 1.7 ± 0.8. Less than one third took OTC medications and more than two thirds took POM. Among the OTC and other medications that were self-administered, the most common were analgesics and antipyretics (42.3%), followed by medications for the leukorrhea (27.8%), alimentary tract and metabolism (12.6%) and antibiotics (8.9%). PW mostly did so on their own decision (59.75%). With the exception of supplements, SM was used more in undergraduates than in graduates (P = 0.05). Including supplements, there was significantly higher use of drugs among graduates, than in undergraduates (P < 0.05). More percentage of graduates advocated the use of iron or folic acid or calcium supplements during pregnancy, than in undergraduates (P < 0.001).

Conclusions: SM, in particular the use of supplements, is more frequent in graduate than in undergraduate PW probably due to higher socioeconomic class and better education about supplements’ benefit and potential risks of other drugs.

Keywords: self-medication, pregnant women, education level.

P274
LITERATURE PATTERN ON OFF-LABEL DRUG USE

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Introduction: ‘Off-label’ drug use is the prescription of medications outside their licensed indications in the terms of dosage, age, indication or route of administration. It is a common practice, especially in neonates and children as well as for diseases that are difficult to treat. This study has been undertaken to examine the pattern and characteristics of literature on off-label drug use.

Methods: A search was conducted on EMBASE and MEDLINE via Ovid SP (from 1988 and 1948 to March 2011, respectively) which was based on two highly sensitive search strategies for retrieving literature on off-label use of medications. Retrieved documents were categorized by research topics, literature characteristics, study population and the names of off-label drugs or unlicensed indications. Anatomical Therapeutic Chemical (ATC) codes and International Statistical Classification of Diseases and Related Health Problems (ICD-10) were used for classification.

Results: A total of 4067 documents were found, published over a 42-year period. The number of documents increased markedly during the period 2003–2010, with an average growth rate of 34.3% per annum. The most popular language of literature on off-label use of medication was English (85.2%). Original (40.8%) and review articles (29.5%) were the most common types of publication. Two thousand five hundred and thirteen documents were classified as preclinical and clinical studies, 513 documents were identified as utilization of off-label drugs and 1041 documents were related to legal, ethical, economic or public health aspects of prescribing drugs beyond their licensed indications. In all categories, the majority of studies had been done in the general population (82.0%). Preclinical and clinical studies were sorted to four subcategories: effectiveness/efficacy studies (58.9%), safety studies (55.1%), clinical application studies (24.4%), and animal/laboratory studies (3.5%). Based on ATC classification, the most frequent topic of preclinical and clinical studies was off-label use of antineoplastic and immunomodulating agents (30.5%). However, according to ICD classification, diseases of the nervous system (19.5%), unlicensed indication for approved drugs, were reported most frequently.

Conclusion: The present study demonstrates an increasing trend of publications on off-label drug use, particularly in the recent years.

P275
UTILIZATION OF NON-STEROIDAL ANTI-INFLAMMATORY DRUGS IN THE OUTPATIENT SETTINGS IN THREE MUNICIPALITIES IN VOJvodina, Serbia

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Introduction: Non-steroidal anti-inflammatory drugs (NSAIDs) are used in the treatment of the musculoskeletal system diseases (MSD) and their use is associated with numerous adverse effects. The aims of this study were to determine the amount and structure of the outpatient consumption of NSAIDs and its correlation with the MSD in three municipalities in Vojvodina with different levels of health care and to examine whether the cost of drugs and the level of health care influence the choice of drug.

Materials and Methods: Data on quarterly consumption of NSAIDs have been collected from all private and state-owned pharmacies in Novi Sad, Vrbas and Backa Palanka. The data are presented as number of DDD/1000 inhabitants/day. Cost/DDD was also calculated. The DU90% methodology was used. Data on the incidence of the musculoskeletal system disease were obtained from the Institute of Public Health of Vojvodina.
Results: The highest total outpatient consumption of NSAIDs was recorded in Backa Palanka, where the highest prevalence of MSD was also observed. The lowest NSAIDs consumption and the number of patients were observed in Novi Sad. Considering the structure of consumed NSAIDs in all three municipalities, diclofenac was in the first place, while ibuprofen, naproxen, meloxicam and nimesulide were dispensed in much smaller amounts. Among the cheapest NSAIDs are piroxicam and naproxen, while diclofenac is in the third place and ibuprofen in the sixth.

Conclusion: In order to improve both prescription practice, and the profile of used drugs in the population, it is important to continuously educate health care professionals, as well as to inform general population about the risks of inappropriate drug use.

Acknowledgements: This research is part of the project No. 41012 which is financially supported by the Ministry of Science of the Republic of Serbia.

Keywords: ATC/DDD methodology, NSAIDs, Pharmacoepidemiology, Serbia.

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OUT HOSPITAL USE OF ATC GROUP N DRUGS FUNDED BY HEALTH SERVICE FUND IN MONTENEGRO IN 2009
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Analysis of out hospital drug use ATC-group N drugs given on prescription funded by Health Service Fund (HSF) of Montenegro was based on data taken from the Informational system of the HSF of Montenegro for the year 2009. Total outpatient drug use funded by HSF in Montenegro in 2009 was 291.05 DDD/1000 inhabitants/day. Drugs for nervous system (group N), were on the second place according to amount used with 3124 DDD/1000 inh/day or 10.73%. Fourth largest amount of money was used for this group (1.749.52671€).

In the group N the most frequently used subgroup are psycholeptics (subgroup N05) with 1955 DDD/1000 inh/day or 62.58% of total in group N. As for financial resources used, this subgroup also takes first place with 599.549.72€ or 34.27% of total expended finances in this group in 2009. In subgroup N05 on the first place according to amount of DDDs spent are anxiolytics with 16.09 DDD/1000 inh/day or 82.30%, on the second place are antipsychotics with 2.82 DDD/1000 inh/day or 14.42%, while on the third place are hypnotics and sedatives with 0.64 DDD/1000 inh/day or 3.27% of total drug utilization inside this subgroup. Fundings spent on anxiolytics are 250.317.07€ or 41.75%, on antipsychotics, 331.446.93€ or 55.28%, and on hypnotics and sedatives 17.785.72€ or 2.97% of total finances spent for subgroup N05 in the year 2009. In comparison to year 2007, use of drugs of group N is increased for 1.99 DDD/1000 inh/day or 6.80%. Fundings spent for this group of drugs increased. This Research was Financially Supported by Ministry of Science, Republic of Serbia, Project NO 41012.

P277
COMPARISON OF OUTPATIENT CONSUMPTION OF ANTIASTHOMATIC DRUGS IN TWO MUNICIPALITIES IN SOUTH BACKA DISTRICT, SERBIA
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Introduction: Asthma is respiratory disease of considerable importance due to its high morbidity and mortality rates. However, little is known about actual antiasthmatic drug consumption in Serbia. The aim of the study was to compare the outpatient consumption of antiasthmatic drugs in two municipalities in the same district – Novi Sad (323.708 inhabitants) and Vrbas (43.840 inhabitants).

Materials and Methods: Data on number of packages and size of packages of antiasthmatic drugs (ATC group R03) from 1 January to 31 March 2008 were obtained from all state-owned and private pharmacies in these municipalities in South Backa District, Serbia. Consumption was expressed as DDD/1000 inh/day that was calculated using ATC/DDD methodology.

Results: The total consumption of R03 group of drugs was higher in Novi Sad (32.57 DDD/1000 inh/day) than in Vrbas (27.89 DDD/1000 inh/day) which was lower than in other European countries. The most frequently used drugs in Novi Sad were inhalants – combination of fenoterol and ipratropium (13.09 DDD/1000 inh/day) and beclometasone (7.15 DDD/1000 inh/day). These drugs were also most frequently used drugs in Vrbas, but their consumption was lower. Interestingly, the consumption of new, expensive group of drugs leukotriene receptor antagonists was low in both municipalities.

Conclusions: These results of consumption of antiasthmatic drugs in South Backa District shows that there is a pharmaco economical impact on a structure of drugs used in asthma therapy.

Acknowledgement: This research is part of the project No. 41012 which is financially supported by the Ministry of Science of the Republic of Serbia.

P278
IS THERE A DIFFERENCE OF BENZODIAZEPINES USE BETWEEN URBAN AND RURAL COMMUNITIES?
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Introduction: Benzodiazepines are widely used in clinics and for recreational purposes, but there is little information about difference of their use between urban and rural communities. The aim of the study was to compare the outpatient consumption of benzodiazepines in urban community-Novi Sad (323.708 inhabitants) and rural-Selcena (3279 inhabitants) in South Backa district, Serbia.

Materials and Methods: Data on number of packages and size of packages of benzodiazepine (ATC group N05) from 1 January to 31 March 2008 were obtained from all state-owned and private pharmacies in these municipalities. Consumption was expressed as DDD/1000 inh/day that was calculated using ATC/DDD methodology.

Results: The total consumption in South Backa District was higher than in Scandinavian countries. In Novi Sad the consumption of N05 group of drugs was significantly higher (68.59 DDD/1000 inh/day) than in Selcena (39.46 DDD/1000 inh/day). The most frequently used drugs in Novi Sad were diazepam (27.09 DDD/1000 inh/day), bromazepam (16.10 DDD/1000 inh/day) andlorazepam (10.99 DDD/1000 inh/day). However, the consumption of non-benzodiazepine hypnotic (zolpidem) in Novi Sad was lower than Scandinavian countries, while there was no consumption of this drug in Selcena.

Conclusions: The consumption of benzodiazepines in South Backa District is higher than in Scandinavian countries. The way of life does have impact on the use of benzodiazepines.

Acknowledgement: This research is part of the project No. 41012 which is financially supported by the Ministry of Science of the Republic of Serbia.

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SELF-MEDICATION AMONG HEALTHCARE STUDENTS IN MONTENEGRO: DOES EDUCATION MAKES ANY DIFFERENCE?
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Introduction: There are no relevant data about self-medication in students population in Montenegro. The aim of this pilot study was to...
determine the prevalence of self-medication and the influence of medical training on the knowledge, attitude and behavior of medical students.

**Materials and Methods:** An anonymous questionnaire consisted of 29 open-ended and close-ended items (demographic data, frequency and reasons for self-medication and types of drugs used for that purpose) was administered to the medical students in their 3rd year of medical course (n = 38) at their first Pharmacology lecture and to the 6th year students (n = 31) at their last lecture of Clinical Pharmacology. Data were statistically analyzed and results were expressed as percentages. Chi-square-test was applied and P < 0.05 was considered significant.

**Results:** The prevalence of self-medication was 94.7% among 3rd year students compared to 77.4% among 6th year students (P > 0.05). There was significant difference (P < 0.05) between 3rd and 6th year students about the most common reason for self-medication: 68.4% of 3rd year students marked previous experience with drug and 41.9% of 6th year students the opinion they know enough about the drug they are taking. The most common reasons for self-medication in both groups were headache, flu and menstrual pain. Commonly used medicines were analgesics (3rd year: 84.2%, 6th year: 67.7%), antibiotics (3rd year: 42.1%, 6th year: 16.1%) and vitamins and minerals (3rd year: 34.2%, 6th year: 19.3%). The most popular analgesics among 3rd year students were ibuprofen (39%), metamizol (18.6%) and paracetamol in combination (16.9%) and among 6th year students- ibuprofen (38.1%), diclofenac (21.4%) and paracetamol (19%).

**Conclusion:** Prevalence of self-medication among students of Faculty of Medicine in Montenegro is higher than in countries in region. There is a need to educate the students about the potential short and long-term side effects and differences between drugs to ensure safer practice.

**Keywords:** self-medication, students, education.

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**P280 CHANGES IN THE USE OF ANTIBIOTICS IN AMBULATORY CARE AMONG ESTONIAN CHILDREN UNDER 4 YEARS OF AGE, 2001–2007**

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Misure of antibiotics, one of the major determinants in development of resistance, is prevalent especially in children. Respiratory infections are the main reason for an antibiotic prescription, although most of these are of viral origin.

The aim of the study was to analyse the use of antibiotics in the treatment of children under 4 years during the period of 2001–2007. The data were obtained from the Estonian Health Insurance Fund database containing all prescriptions of reimbursed medicinal products. The data of 2001, 2005 and 2007 were used.

Antibiotics made up 40% of all prescriptions for children, this proportion was higher in 2001 compared to the later years (44%, 39% and 38%). 2/3 of children received at least one prescription of antibiotics per year, in 2005 the number was lower (57%).

The most often prescribed antibiotic was amoxicillin. Erythromycin and combination of trimethoprim and sulfamethoxasol ranked second and third in 2001. Six years later trimethoprim plus sulfamethoxasol was still in top five, erythromycin had been replaced by clarithromycin. Use of cephalosporines and amoxicillin plus clavulanic acid had increased. Penicillin was used only in negligible quantity during the study period.

Antibiotics were the most often prescribed medicines for children >4 years of age and broad-spectrum penicillines were the first choice during the period 2001–2007. There were limited changes in the total use of antibiotics, the number of prescriptions increased slightly and the number of children treated with antibiotics decreased by 5%. The change in drug selection was more marked.
P283
HOSPITAL USE OF DRUGS IN UROLOGY CLINIC OF CLINICAL CENTRE OF MONTENEGRO
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Introduction: Drug utilization analysis is an important segment of pharmacotherapy, because it can point out the need and the ways of therapy rationalization.

Materials and Methods: We used Central Pharmacy Report as the source of data about drugs delivered to Urology Clinic in the period of time January 1–September 30, 2004, and in the same period in the year 2010. Total consumption rate was calculated as the number of daily defined doses (DDD), used in 100 bed days (BD). The drugs were classified according to the ATC classification.

Results: The total amount of drug consumption was 477.27 DDD/100 BD for the year of 2004, or 473.91 DDD/100 BD for 2010, which means that every hospitalized patient was getting 4.7 drugs in average during 1 day of treatment. Based on the results from 2004 and 2010, as the most present drug group, the B group by ATC classification stood out, that is the drugs used in treatment of blood and blood forming organs dysfunctions (52.1%, 39.4%). On the second place are the antifungicins for systemic use (ATC group J) with 13.8% in 2004, or with 17.4% in 2010, while the third place goes to alimentary tract and metabolism drugs (ATC group A) with the percentage of 10.6% and 11.6%. The largest consumption of antibiotics belong to cephalosporins because the consumption of them in 2004 and 2010 is over 50%.

Conclusion: Utilization of analyzed drug groups in majority concords with contemporary pharmacotherapy principles.

P284
USE OF DRUGS IN ELDERLY PATIENTS FROM GERONTOLOGY CENTER NOVI SAD: A DESCRIPTIVE STUDY
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Introduction: If we take a look on Serbia, we can see that population >15 years of life is already almost equal to the number of people older than 65 years. According to this indicator, our country is the fourth in Europe in terms of morbidity and mortality leading to death. The number of people older than 65 years is larger than the number of the people older than 15 years. In the world, the most rapid increase in population is expected in the group of people 60 years and older. The number of people older than 65 years will exceed the number of people younger than 15 years of age by 2030. The number of people older than 80 years of age is expected to increase by four times. Older persons have many medical problems which require prescription of multiple drugs, and that leads to polypharmacy, or ‘problems associated with drugs’. Bearing in mind these problems our aim was to gain insight into the morbidity of the treated population and structure of drugs for diseases of the respondents.

Methods: After receiving approval of the Ethical Committee of the Faculty of Medicine in Novi Sad research has been conducted from January through June 2010 year. Data were collected in the Gerontology Center of Novi Sad. When patients have signed the informed consent, we accessed the data collection. The data were obtained by interview, using a standardized questionnaire, as well as by insight into the inventory of drugs that the respondents possessed. The survey was conducted through interviews face-to-face, reading and filling out the questionnaire by the examiner, and the adequacy of therapy was evaluated by reading medical records of patients.

Results: Of the total number of respondents, 9% do not regularly take any medication for the treatment of chronic diseases that are prescribed by their doctor, while the number of patients who regularly take at least one drug is 91%. The number of drugs which patients were taken regularly, ranged from 1 to 10, and at times from 1 to 6 drugs. It was also observed that no one take all prescribed medications regularly. More than 4/5 patients, precisely 81.93% had a simultaneous diagnosis of two or more chronic diseases. Most of our patients had, as expected, some of the cardiovascular diseases (52.65%). Next most common were locomotion disorders (11.42%), followed by metabolic and gastrointestinal (11.69%) and central nervous system (CNS) diseases (10.31%).

Conclusion: In this population, there are many examples of inadequate treatment of disease in terms of hypomedication, hypermedication or polypharmacy.

P285
PHARMACOTHERAPEUTIC TREATMENT OF ATTENTION-DEFICIT/HYPERACTIVITY DISORDER IN CHILDREN AND ADOLESCENTS
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Introduction: The ideal treatment of Attention-Deficit/Hyperactivity Disorder is highly debated. Opposition to pharmacological treatments has resulted in the popular use of non-pharmacological measures which are not necessarily efficacious for ADHD. The primary aim of the study was to evaluate the treatment of children and adolescents diagnosed with ADHD in South Africa.

Methods: The study consisted of a drug utilisation review (DUR) and questionnaire-based surveys. The DUR was conducted using a 2008 medical aid administrator database. The primary study population constituted 21,650 prescriptions dispensed to 7202 patients. Questionnaires were distributed to the parents/caregivers of children diagnosed with ADHD. The response rate was 20.81%.

Results: The average age of DUR patients 11.60 ± 3.01 years. Male patients represented 74.09% (5336: n = 7202) of patients. Methylphenidate was the most commonly prescribed of the two drugs indicated for the treatment of ADHD (85.89%). A prescribing bias by practitioners in different parts of South Africa was identified. Drug holidays were identified during March and December 2008. The average age of patients in the questionnaire was 10.67 ± 2.83 years, with a male patient majority (86.11%). Methylphenidate was the most commonly used prescription treatment (93.75%). Drug holiday use was reported in 56.25% of patients. Most participants reported supplement use (83.33%), but 86.67% of these did not find them useful.

Conclusion: ADHD is a poorly understood disorder which affects people in all spheres of life. ADHD treatment should be individualised and based on scientifically proven effectiveness. Further studies need to be conducted on the treatment of ADHD.
PHARMACOECONOMICS

P286
THE QUESTION OF TRANSPARENCY IN DRUGS PURCHASING PROCESS WITHIN THE BOUNDS OF ON-BUDGET EXPENDITURES OF HEALTH-CARE SYSTEM

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Objective: To analyze transparency in drug purchasing system and to provide offers for its increase (research had the support of The Soros Foundation – Kazakhstan and Open Society Institute).

Materials and Methods: Interviewing with questionnaire method of employees medico-prophylactic foundings, which take part in formulation of Log-book list and buying drugs at social-meaning diseases: oncopathology (including haemoblastoses), hemophilia, tuberculosis, diabetes.

Results: In work a special attention was given to formation of drug names list which are awaiting purchase, tendering process and procedures of authorized bodies which purchase drugs within the bounds of on-budget expenditures. Results of the analysis have shown, that the scheme of formation of drug names list: selection of applications for drugs from medical institutions and preparation list of drugs which are awaiting purchase at the uniform distributor ‘SK-pharmacy’ – practically is inaccessible and is not transparent at all stages. The tender for drugs purchase is spent by the uniform distributor basically on basis of price offers nonmetering quality of preparations. The system of limit drug prices formation within the bounds of Guaranteed Medical Service is not clear at all, the normative legal document adjusting this questions works not effectively. The legislation does not take a measure preventing generics purchasing without proofs of therapeutic equivalence.

Conclusion: According to research with a view to perfection of purchasing system have been offered appropriating recommendations.

P287
AN OPTIMIZATION MODEL TO REDUCE TPA EXPENDITURE IN STROKE EVENTS

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According to 2010 Israeli survey, only 5.9% of patients received tPA treatment in stroke events. tPA’s preparation by physicians is based on axioms as: standard dose, urgent IV preparation before procedure, quantity of drug calculated by body weight and short stability of reconstituted vial. Due to those limitations, wastage cost, is high. Study’s aim is to optimize use of tPA and reduce drug expenditure.

A model of optimization, based on credibility and prediction tests was used. It uses parameters such as number of patients receiving tPA, quantity of drug dispersed, used and wasted. The model identifies wastage (quantities and cost) and predicts optimal dose and quantities to be prepared. Pharmacists prepared standard dosage syringes with a 6 month stability (frozen). Physicians administer patients tailored dosage, using a combination of vial and pharmacy pre-prepared injections.

The model predicts a 10 mg dose of tPA as the optimal one. Wastage of tPA was calculated for method A (preparation by the physician) and B - (using a combination of vial and pre-prepared injections). During a 2 years period, wastage using method B was 3.6% (mg) vs. method A 31.8%. The outcome was a 101.285 $ savings from pharmacy budget. This optimization model can be applied for costly drugs and high wastage rate. It will enable lowest wastage with minimal addition of manpower.

P288
USE OF ACE INHIBITORS IN SERBIA COMPARED WITH MONTENEGRO IN 2009: PHARMACOEPIDEMIOLOGY AND PHARMACOECONOMIC ASPECTS

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The aim of the study was to analyze use of ACE inhibitors (subgroup C09) in Serbia compared with Montenegro in year 2009.

Use of ACE inhibitors in Serbia in 2009 was 179.26 DDD/1000 inh/day. In 2009 year use of ACE inhibitors in Montenegro was 8332 DDD/1000 inh/day. In Serbia 5.977.289,00€ has been spent for ACE inhibitors in 2009, while in Montenegro 2.488.464,95€ in the same year.

Comparing the consumption of ACE inhibitors in Serbia and Montenegro in year 2009.

The consumption of ACE inhibitors in the subgroup C09 is most frequently used in Montenegro, while in Serbia the use of this combination is on the fifth place in this group of drugs.

This research was financially supported by Ministry of Science, Republic of Serbia, Project NO 41012.
PK/PD RELATIONSHIP

P289
RITONAVIR INHIBITS PRASUGREL IN VITRO BIOACTIVATION: A POTENTIAL PHARMACOKINETIC INTERACTION
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Background: Prasugrel is an antiplatelet prodrug used in patients with acute coronary syndrome. Prasugrel is mainly bioactivated by CYP3A and CYP2B6. HIV patients are at risk of cardiovascular disease and pro-tease inhibitor such as ritonavir is also a potent inhibitor of these two CYP and could therefore impaired clopidogrel efficacy. The aim of this in vitro study was to evaluate the potential interaction between ritonavir and prasugrel using human liver microsomes (HLM).

Methods: HLM and recombinant CYP microsomes were used to identify the CYP enzyme responsible for the bioactivation of prasugrel. Prasugrel concentrations of 5, 20, 40, 80, and 200 μM were used for Km determination. Inhibition by ritonavir was characterized using HLM and at concentrations of 0.1, 1, 5, 10, 15, 30 μM. Prasugrel metabolite determination was performed using a validated LC/MS method.

Results: Using recombinant CYP microsomes, the prasugrel biotransformation was produced mainly by CYP2B6 (Km = 1.2 μM), CYP2D6 (Km = 16 μM), CYP2C19 (Km = 35 μM) and CYP3A4 (Km = 88.9 μM), CYP3A5 (Km = 85 μM). In the presence of specific inhibitors of CYP3A, 2B6, 2D6, 2C9 and 2C19, the active metabolite production was decreased by 38 ± 15% in presence of 4-(4-chlorobenzyl) pyridine (CYP2B6 inhibitor) and 45 ± 16% with ketoconazole (CYP3A inhibitor). The other inhibitors did not reduce significantly metabolite production. The Km value for prasugrel metabolism in human liver microsomes was determined at 92.5 μM. Ritonavir from 0.1 to 30 μM was shown to be a dose-dependent potent inhibitor for each prasugrel concentration tested (20, 50, 100, 200 μM).

Conclusion: Prasugrel is mainly bioactivated by CYP3A4/5 and CYP2B6. Inhibition of biotransformation of prasugrel by ritonavir suggests a potential clinically significant drug-drug interaction between these two drugs.

P290
THE ABSOLUTE BIOAVAILABILITY AND CLINICAL PHARMACOKINETICS AFTER INTRAVENOUS AND ORAL ADMINISTRATION OF THE NOVEL ANTIDEPRESSANT LU AA21004
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Introduction: Lu AA21004 is a novel compound under development as a multimodal antidepressant. In recombinant cell lines, Lu AA21004 is a 5-HT3 and 5-HT7 receptor antagonist, 5-HT1A receptor agonist, 5-HT1B receptor partial agonist and inhibitor of the 5-HT transporter. The compound is currently under phase III development. Lu AA21004 is cleared through the liver by oxidation and glucuronic acid conjugation. The purpose of the study was to determine the absolute bioavailability of Lu AA21004 and to determine the pharmacokinetic parameters of Lu AA21004 and its metabolite Lu AA34443 after intravenous (i.v.) administration and oral delivery of Lu AA21004 to healthy subjects.

Methods: Twenty-two healthy subjects (11 men, and 11 women, mean age 35 years, mean weight 75 kg and mean BMI 25 kg/m²) were first given 20 mg Lu AA21004 administered as a single oral dose of 2 x 10 mg IR (immediate release) tablets. Eighteen days later, the same subjects were given a single dose of 10 mg Lu AA21004 as an i.v. infusion over 6 h. Venous blood samples (3 ml) for analysis of plasma concentrations of Lu AA21004 and Lu AA34443, using validated methods according to Food and Drug Administration (FDA) Guidance for Industry, were drawn at 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 16, 24, 36, 48, 72, 96, 120, 144, 168, 192, and 240 h post-dose (oral dosing) and at 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 8, 10, 12, 16, 24, 36, 48, 72, 96, 120, 144, 168, 192, and 240 h post-start-of-infusion (i.v. dosing). Pharmacokinetic parameters, including absolute bioavailability, were estimated with standard non-compartmental analysis methods using WinNonlin software, version 5.2.

Results: The absolute bioavailability of Lu AA21004, based on the individual dose-normalised AUC(0-inf) ratios, following a single oral dose of 20 mg and a single i.v. infusion at a dose of 10 mg was 0.749 (95% confidence interval [0.713, 0.788]). For individual subjects, the observed absolute bioavailability ranged between 0.646 and 0.940, with an inter-subject variability (CV%) of 12%. The mean systemic clearance (CL) was 26.0 l/h (SD = 8.2 l/h), while the mean volume of distribution (Vz) was 2420 l (SD = 535 l). The mean elimination half-life for Lu AA21004 was 68.9 h (SD = 21.7 h) after i.v. administration and 69.1 h (SD = 20.0 h) after oral administration. The metabolic ratio [AUC(0-inf)] ratio between Lu AA34443 and Lu AA21004 was 0.938 after oral dosing and 0.704 after i.v. dosing, clearly indicating the presence of post-systemic metabolism.

Conclusion: The absolute bioavailability of Lu AA21004 was 75%.

P292
INVESTIGATION OF PHARMACOKINETIC AND PHARMACODYNAMIC INTERACTIONS BETWEEN CLAZOSENTAN AND NIMODIPINE IN ASAH PATIENTS: A POPULATION ANALYSIS
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¹Aelction Pharmaceuticals Ltd, Allschwil, Switzerland; ²EMF Consulting, Aix-en-Provence, France

In this phase 3 study 1147 aneurysmal subarachnoid haemorrhage (aSAH) patients, secured by surgical clipping, were infused with clazosentan 5 mg/h, a selective endothelin A (ETA) receptor antagonist, or placebo until Day 14 post-aSAH. The aim of this analysis was to investigate PK/PD interaction potential between nimodipine, a calcium channel blocker currently used in the treatment of aSAH patients, and clazosentan. Administration of oral nimodipine according to routine standard procedures (30 or 60 mg q 4 h) was permitted in the study. The population PK analysis of clazosentan and nimodipine, and clazosentan population PK/PD analyses of diastolic/systolic blood pressure (DBP/ SBP) and mean arterial pressure (MAP) were based on non-linear mixed effect modelling using NONMEM. The PK/PD population included 1021 patients. In the PK/PD model effect of nimodipine co-administration as a covariate was determined. Clazosentan clearance (CL) was 1021 patients. In the PK/PD model effect of nimodipine co-administration as a covariate was determined. Clazosentan clearance (CL) was 34.4 l/h. The volume of distribution (V) was 24.3 l and 34.9 l in females and males, respectively. There were no statistically significant effects of clazosentan on the PK of nimodipine and vice versa. The median clazosentan plasma concentrations on 3rd and 9th day of infusion were 141 and 126 ng/ml, respectively. Blood pressure decreased proportionally with clazosentan concentration with DBP, SBP, and MAP decreasing by 0.020, 0.028, and 0.025 (mmHg), respectively, per 1 ng/ml of clazosentan. Nimodipine did not amplify this decrease in blood pressure. Collectively, the data suggest that there are no pharmacokinetic or pharmacodynamic interactions between clazosentan and nimodipine.
P293
POPULATION PHARMACOKINETIC AND PHARMACODYNAMIC ANALYSIS OF LIPID EMULSION PROPOFOL IN CHILDREN
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Background: The aim of this study was to characterize pharmacokinetics and pharmacodynamics of lipid emulsion propofol in children.

Methods: Forty patients (ASA PS 1, 2) aged 2–12 years were given an intravenous bolus of 2% propofol (Fresofol®, Fresenius Kabi Korea Ltd., Korea) at a dose of 3 mg/kg, followed by continuous infusion at the rate of 0.2 mg/kg/min for variable periods. Arterial concentrations of propofol were measured at preset intervals and bispectral index (BIS) values were recorded throughout the study period. Pharmacokinetic and pharmacodynamic characteristics were evaluated using a population analysis with nonlinear mixed effects modeling.

Results: Pharmacokinetics and pharmacodynamics of propofol in children were best described by a two compartment model and inhibitory effect-compartment model, respectively. Population parameter estimates, inter-individual variability, and median parameter values (2.5–97.5%) of the non-parametric bootstrap replicates of the final pharmacokinetic and pharmacodynamic models are shown in Tables 1 and 2, respectively.

Table 1. Population parameter estimates, inter-individual variability, and median parameter values (2.5–97.5%) of the non-parametric bootstrap replicates (B = 2000) of the final pharmacokinetic model of propofol in children (n = 39)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Estimates</th>
<th>SE</th>
<th>CV (%)</th>
<th>Median</th>
<th>2.5%</th>
<th>97.5%</th>
</tr>
</thead>
<tbody>
<tr>
<td>V1 (L)</td>
<td>1.7</td>
<td>0.5</td>
<td>84.9</td>
<td>2.1</td>
<td>1.4</td>
<td>2.4</td>
</tr>
<tr>
<td>V2 (L)</td>
<td>323.5</td>
<td>1.5</td>
<td>19.8</td>
<td>22.2</td>
<td>20.4</td>
<td>26.1</td>
</tr>
<tr>
<td>Cl (l/min)</td>
<td>0.381</td>
<td>0.041</td>
<td>30.7</td>
<td>0.04</td>
<td>0.03</td>
<td>0.04</td>
</tr>
<tr>
<td>Q (l/min)</td>
<td>1.3</td>
<td>0.38</td>
<td>88.9</td>
<td>1.3</td>
<td>0.1</td>
<td>1.7</td>
</tr>
<tr>
<td>Residual error (%)</td>
<td>26.9</td>
<td>1.71</td>
<td>25.6</td>
<td>22.5</td>
<td>29</td>
<td></td>
</tr>
</tbody>
</table>

Log-normal distribution was assumed for inter-individual random variability. Residual random variability was modeled using a constant coefficient of variation (CV) model.

*Body weight in kg.

Table 2. Population parameter estimates, inter-individual variability, and median parameter values (2.5–97.5%) of the non-parametric bootstrap replicates (B = 2000) of the final pharmacodynamic model of propofol in children (n = 39)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Estimates</th>
<th>SE</th>
<th>CV (%)</th>
<th>Median</th>
<th>2.5%</th>
<th>97.5%</th>
</tr>
</thead>
<tbody>
<tr>
<td>E0</td>
<td>80.8</td>
<td>2.0</td>
<td>–</td>
<td>81.2</td>
<td>79.6</td>
<td>83.2</td>
</tr>
<tr>
<td>Emax</td>
<td>38.6</td>
<td>1.89</td>
<td>22.4</td>
<td>35.4</td>
<td>25.2</td>
<td>40.7</td>
</tr>
<tr>
<td>Ce50 (?/ml)</td>
<td>1.9</td>
<td>0.4</td>
<td>80.6</td>
<td>2.3</td>
<td>1.5</td>
<td>3.0</td>
</tr>
<tr>
<td>r</td>
<td>2.9</td>
<td>0.3</td>
<td>–</td>
<td>2.4</td>
<td>1.5</td>
<td>3.7</td>
</tr>
<tr>
<td>Ke0 (l/min)</td>
<td>0.45</td>
<td>0.1</td>
<td>57.5</td>
<td>0.4</td>
<td>0.3</td>
<td>0.5</td>
</tr>
<tr>
<td>r2</td>
<td>76</td>
<td>1.1</td>
<td>–</td>
<td>75</td>
<td>59</td>
<td>158</td>
</tr>
</tbody>
</table>

Inter-individual random variability of Emax was modeled using an additive error model. Log-normal distribution was assumed for Ce50 and Ke0. No inter-individual random variability was assumed for E0 and r. Residual random variability was modeled using an additive error model. CV, coefficient of variation.

Conclusions: In children, body weight was a significant covariate for V2 and Cl. The blood-brain equilibration half-time was 1.54 min for BIS.

P294
RELATIONSHIP BETWEEN CORTICOSTEROIDS AND CONCENTRATION OF TACROLIMUS AND BIOCHEMICAL STATUS IN RENAL TRANSPLANT RECIPIENTS
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Introduction: Tacrolimus is standard immunosuppressive drug in many kidney transplant centres. Monitoring of tacrolimus concentration is of utmost importance due to its narrow therapeutic index, variable pharmacokinetics and interactions with medicines. Corticosteroids are inducers of CYP3A4 enzyme which participates in metabolism of tacrolimus so that their influence on tacrolimus concentration has clinical importance.

Aim: To evaluate the effect of corticosteroids on tacrolimus pharmacokinetics and biochemical parameters in renal transplant recipients, in the early post-transplantation period.

Patients and Method: We studied 35 kidney transplant patients, on triple immunosuppressive therapy treated in Clinical Centre of Nis, Serbia, during 2009–2010. Kidney transplant recipients were treated with tacrolimus and mycophenolate mofetil with 1 months of prednisone. Patients were divided into three groups, according to tacrolimus dose (I: <0.10 mg/kg/day; II: 0.10–0.17 mg/kg/day and III: >0.17 mg/kg day). Tacrolimus blood concentrations were measured by microparticle enzyme immunoassay method (IMx, Abbott). The obtained results were processed with the statistic student’s t-test and Mann–Whitney U-test.

Results: The commonest applied doses of tacrolimus were in the range of 0.10–0.17 mg/kg/day. During the study it was determined that the steroid dose reduction (from 1.63 to 0.46 mg/kg/day) was followed by the increase tacrolimus concentration/dose ratio, after the transplantation. Out of biochemical parameters, it was established that corticosteroids affect the levels of creatinine and cholesterol.

Conclusion: We demonstrated that interaction occurs between steroids and tacrolimus and biochemical parameters in kidney transplant patients. Monitoring of tacrolimus concentration during steroid sparing must be taken into account because it may be associated with tacrolimus-related nephrotoxicity.

P295
IMPACT OF SERUM ALBUMIN LEVEL ON FREE OXYCODONE PHARMACOKINETICS, DOSE ESCALATION, AND CENTRAL ADVERSE REACTION IN CANCER PATIENTS
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Introduction: Serum albumin level decreases owing to the under-nutrition in cancer patients. In contrast, serum inflammatory protein such as α1-acid glycoprotein (AGP) was secreted with spreading the invasive cancer cells. It remains to be clarified whether or not these serum proteins influence the oxycodone pharmacokinetics and clinical responses. The aim of this study was to evaluate the influences of serum albumin and AGP levels on free oxycodone pharmacokinetics, dose escalation, and central adverse reaction (CAR) in cancer patients.

Methods: Fifty-one Japanese cancer patients receiving oxycodone extended-release tablets were enrolled. Predose total and free plasma concentrations of oxycodone were determined by HPLC-MS/MS. Daily oxycodone escalation rate was evaluated as the opioid escalation index (OEI). The CAR included drowsiness and confusion.

Conclusion: We demonstrated that interaction occurs between steroids and oxycodone and biochemical parameters in kidney transplant patients. Monitoring of tacrolimus concentration during steroid sparing must be taken into account because it may be associated with tacrolimus-related nephrotoxicity.
dence of CAR. In contrast, the serum AGP level did not influence the OEL and incidence of CAR.

Conclusion: The serum albumin but not AGP level altered the free fraction of oxycodone in cancer patients. In addition, the serum albumin level influenced the dose escalation and CAR of oxycodone in this study population.

P296
PK/PD MODELING OF DIURNAL INTRAOCULAR (IOP) FLUCTUATIONS IN GLAUCOMA PATIENTS
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Introduction: Brinzolamide and brimonidine are used to treat elevated IOP associated with open-angle glaucoma and ocular hypertension. They are also prescribed concomitantly for improved disease management. This PK/PD analysis characterizes the diurnal fluctuation of IOP and assesses the IOP-reducing effects of brinzolamide and brimonidine administered alone and in combination.

Patients and Methods: IOP measurements were available from 2439 patients in seven Phase 2 & 3 studies. Patients received brinzolamide alone, brimonidine alone, and in combination. A population pharmacodynamic model was developed to describe the IOP at baseline (before drug treatment) and a KPD model to describe the drug effect of IOP reduction. Direct and indirect response models with either linear or sigmoidal (E\text{max}) models were evaluated.

Results: Diurnal fluctuations of the baseline IOP were characterized using a Fourier series model with 12- and 24-h period harmonics. A direct response E\text{max} model was superior to other models evaluated. All model parameters were estimated with adequate precision, with relative standard errors of 20% or less. Interindividual variability ranged between 10% to 35% for all estimated parameters except IC50. High interindividual variability was observed in the IC50 estimates (95–204%) due to the small range of doses used in the studies. The effect of the combination therapy was superior to each of the single treatments, though it appears less than additive.

Conclusion: An integrated KPD model characterized the baseline diurnal fluctuations of IOP and the effect of the agents (alone and in combination) on IOP reduction.

P297
RELATIONSHIPS BETWEEN PROCHLORPERAZINE PHARMACOKINETICS AND ITS CLINICAL RESPONSES BASED ON DRD2 GENE POLYMORPHISMS IN CANCER PATIENTS RECEIVING OPIOID ANALGESICS
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Introduction: Prochlorperazine (PCZ), a dopamine receptor D2 (DRD2) inhibitor, was used for opioid-induced nausea and vomiting. It possesses the interindividual variations in the efficacy and adverse reactions. However, the factors determining these clinical responses have not been fully clarified. The aim of this study was to evaluate the relationships between the PCZ pharmacokinetics and the incidences of nausea and vomiting and serum prolactin level based on DRD2 gene polymorphisms in cancer patients.

Methods: Seventy-one (51 men and 20 women) Japanese cancer patients receiving PCZ with opioid analgesics were enrolled. Plasma concentration of PCZ was determined by HPLC-MS/MS. The incidences of opioid-induced nausea and vomiting were obtained from medical records. Serum prolactin level was determined by CLIA. DRD2 gene polymorphisms of Taq IA, Taq IB, 141C ins/del and A-241G were examined using PCR-RFLP.

Results: The incidence of nausea tended to be higher in DRD2 Taq IB B1B1 + B1B2 group than in B2B2 group (P = 0.057). In contrast, the plasma concentration of PCZ and gender did not influence the incidence of nausea. The plasma concentration of PCZ was slightly correlated with serum prolactin levels (ρ = 0.342, P < 0.01). Following the PCZ treatment, the serum prolactin level in women was higher than that in men (P < 0.01). No significant difference was observed in the serum prolactin level in each DRD2 gene polymorphism.

Conclusion: DRD2 Taq IB gene polymorphism tends to alter the incidence of nausea in cancer patients receiving PCZ with opioid analgesics. In addition, the plasma concentration of PCZ and gender affected the serum prolactin level in this study population.

P298
MYOCARDIAL AND HEPATIC DISPOSITION OF THE ENANTIOMERS OF PERHEXILINE
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Introduction: Perhexilene, an antianginal agent, improves myocardial oxygen utilisation by altering myocardial metabolism. In humans it is cleared primarily by CYP2D6 metabolism, and elevated concentrations in plasma have been associated with hepatic and neuronal toxicity. It is administered as a 50:50 mix of (+)- and (-)-enantiomers, however the hepatic and myocardial disposition of the individual enantiomers is unknown. We investigated the pharmacokinetics of (+)- and (-)-perhexilene in female Dark Agouti rats, which we have previously shown are a model of intermediate metabolism of racemic-perhexilene.

Methods: Animals were dosed with vehicle or 200 mg/kg of racemic-, (+)- or (-)-perhexilene daily for 2 months. At 2 months, blood was withdrawn from the tail vein to assess plasma perhexilene and hydroxy perhexilene concentrations, as well as liver function tests.

Results: The plasma concentrations of (+)- and (-)-perhexilene were within the range attained clinically for all animals, with (+)-clearly evolved more slowly than (-)-perhexilene. Following administration of racemic-perhexilene the mean (SEM) ratios of tissue/plasma concentrations for (+)- and (-)-perhexilene were 53.8 (6.3) and 46.5 (5.1), respectively in liver and 43.2 (8.9) and 34.5 (6.7), respectively in heart. Administration of the pure enantiomers resulted in ratios of 157.0 (20.5) in liver and 74.8 (9.4) in heart following (+)-, and 63.7 (14.0) in liver and 21.2 (3.1) in heart following (-)-perhexilene.

Conclusion: There was extensive accumulation of both enantiomers in heart and liver. Accumulation of (+)-perhexilene was significantly higher (P < 0.01) following administration of pure enantiomer compared to racemate. Better understanding of the pharmacokinetics of the enantiomers may lead to safer and better clinical use of perhexilene.

P299
INTRATHecal GABAPENTIN AND TOCOCHErOL REDUCE THE FORMALIN-INDUCED HYPERALGESIA DOSE DEPENDENTLY IN RAT FORMALIN TEST
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Background and Goal of Study: Gabapentin is thought to appear the effect through the NMDA receptor or voltage-dependent calcium channel. And tocopherol (Vitamin E) is a widely known antioxidant, it neutralize the harmful effect of ROS which is considered to play an important role in various painful conditions. So, this experiment was conducted in order to assess the antinoiceptive effects of gabapentin and tocopherol in the modulation of pain in rats subjected to the formalin test.
Material and Methods: Five percent formalin was injected into the left hind paw after intrathecal administration of either gabapentin or tocopherol dissolved in vehicle or vehicle alone (gabapentin: 10, 30, 100, 300 μg, tocopherol: 1, 10, and 30 mg/kg). The Number of flinches were measured in a 5 min interval for 1 h.

Results and Discussion: Formalin injected into the left hind paw induced a biphasic nociceptive behavior in all rat. Intrathecal injection of gabapentin diminished nociceptive behavior dose dependently during phase 2 but showed no significant difference in phase 1. But, tocopherol diminished nociceptive behavior dose dependently during phase 1 and phase 2.

Conclusion(s): Intrathecal injection of gabapentin and tocopherol produces analgesia in a rat model of formalin-induced hyperalgesia by central sensitization inhibition.

P301
LEVEL B IVIVC DEVELOPMENT FOR EXTENDED-RELEASE SOLID ORAL DOSAGE FORMS
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Introduction: In vitro – in vivo correlation (IVIVC) became a powerful tool for drug manufacturers and regulatory authorities for understanding of the in vivo and in vitro performance of dosage forms. A good IVIVC can allow the use of in vitro dissolution studies for prediction of product in vivo performance.

Materials and Methods: We have studies three extended-release formulations of trimetazidine, indapamide and ciprofloxacin. In vitro dissolution studies were performed using USP Apparatus 2 (Sotax AT7 smart, Allschwil, Switzerland) at 50 rpm. Dissolution media were pH. Eur. 6.0 0.1 M hydrochloric acid solution, acetate buffer solution pH 4.3 and phosphate buffer solution pH 6.8 at 37 ± 0.5°C. All profiles were linearized and summary dissolution parameter, mean dissolution time (MDT in vitro), was calculated. In vivo pharmacokinetics study design was randomized, two-way, crossover, with 14 days washout period, in fasted state, on 18 healthy male and female volunteers. Pharmacokinetic parameters AU0-t, Cmax, Tmax, MRT in vivo, and Cmax/AUC0-t were calculated using KinetikaTM software. In vitro drug release and plasma concentrations were assayed by HPLC with UV-detection (Agilent 1200, Santa-Clara, California).

Results: Level B IVIVC was evaluated by calculation of correlation coefficient (r2) of the mean in vitro dissolution rate (MDT in vitro) and mean in vivo summary parameter (MRT in vivo). r2 for evaluated trimetazidine, indapamide and ciprofloxacin drug products were 0.97, 0.94, and 0.95, respectively.

Conclusion: A good level B IVIVC was developed for evaluated extended-release formulations.

P302
THE INFLUENCE OF FLUCONAZOLE AND CYP2C9*3 ON CYP2C9 ACTIVITY, ASSESSED VIA LOSARTAN URINARY RATIO
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Introduction: Galloping progress in clinical pharmacology allows us to individualize therapy and adjust the dose of drugs as soon as possible. Losartan is selective angiotensin receptor antagonist primarily metabolized by CYP2C9 to the active metabolite E-3174. Losartan is also used to estimate activity of CYP2C9 in vivo and in vitro, which is main passway for warfarine too. We applied losartan probe to estimate the influence of CYP2C9*3 and fluconazole (inhibitor of CYP2C9) on CYP2C9 activity among Moscow population.

Methods: In our study were included 20 healthy volunteers (see Table 1). Study consisted of two stages. At the first stage volunteers took 50 mg of losartan under fasting condition. After 7-days washout period at night before second stage volunteers took 150 mg of fluconazole, next morning they took 50 mg losartin. Eight hour urine portions were collected at each stage of experiment for assessment the losartan urinary ratio (LUR) calculated as (losartan concentration)/(E-3174 concentration). The concentration of losartan and E-3174 were detected by liquid chromatography-mass spectrometry technique. The CYP2C9*2 and *3 polymorphisms were identified using a PCR-RFLP assay. Statistical analysis was conducted in SPSS Statistics 17.0 by means of Wilcoxon criterion.

Results: There was significant LUR increase at the fluconazole stage of study in compassion with first stage (from 15838 [0.50–7.09] to 24255 [0.63–12.03], P = 0.044). After considering the results of a PCR-RFLP, there were none of CYP2C9*2 carriers. Volunteers were divided into two groups: group 1–10 volunteers with CYP2C9*1/1 genotype, group 2 non-*1/1 (CYP2C9*1/3 (8 volunteers) and CYP2C9*3/3 [2 volunteers]). LUR was significantly higher in group 2 at losartan stage (0.8789 [0.50; 2.09] vs. 2.2886 [0.78; 7.09] P = 0.008). At the fluconazole stage there was no significant difference between this groups (1.2340 [0.63; 2.46] vs. 3.6170 [0.74; 12.03] P = 0.089). There was no significant blood pressure decreasing over the time of study.

Table 1. Demographic data of healthy volunteers

<table>
<thead>
<tr>
<th>Volunteers (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, M/W</td>
</tr>
<tr>
<td>Age, years</td>
</tr>
<tr>
<td>High, m</td>
</tr>
<tr>
<td>Weight, kg</td>
</tr>
</tbody>
</table>

Discussion: Results of our study demonstrated the potent inhibitory effect of fluconazole in single dose on CYP2C9. Volunteers among Moscow population with CYP2C9*3 allele have lower CYP2C9 activity in comparison with first stage (from 15838 [0.50–7.09] to 24255 [0.63–12.03], P = 0.044). After considering the results of a PCR-RFLP, there were none of CYP2C9*2 carriers. Volunteers were divided into two groups: group 1–10 volunteers with CYP2C9*1/1 genotype, group 2 non-*1/1 (CYP2C9*1/3 (8 volunteers) and CYP2C9*3/3 [2 volunteers]). LUR was significantly higher in group 2 at losartan stage (0.8789 [0.50; 2.09] vs. 2.2886 [0.78; 7.09] P = 0.008). At the fluconazole stage there was no significant difference between this groups (1.2340 [0.63; 2.46] vs. 3.6170 [0.74; 12.03] P = 0.089). There was no significant blood pressure decreasing over the time of study.

P303
SEASONAL VARIATION IN BLOOD DRUG CONCENTRATIONS AND A POTENTIAL RELATIONSHIP TO VITAMIN D
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Introduction: The most important enzyme in hepatic drug metabolism is cytochrome P450 (CYP) 3A4. Published in vitro data indicate that vitamin D may up-regulate the expression of the CYP3A4 gene. Individual vitamin D-levels are highly dependent on sunlight exposure and show great seasonal variability in northern countries. The aim of the present study was to investigate whether plasma concentrations of CYP3A4 drug substrates exhibit seasonal changes compatible with a stimulatory effect of vitamin D on drug metabolism.

Methods: Three immunosuppressants (tacrolimus, sirolimus, and cyclosporine) were analysed, as these CYP3A4 drug substrates are subject to long-term use and repeated concentration determinations. In addition, mycophenolic acid was included in the analysis as a control drug independent of CYP3A4 metabolism. Concentration-to-dose ratios were extracted from the Karolinska Therapeutic Drug Monitoring database, and compared between the 3-month-periods of lowest and highest vitamin D levels.

Results: Sirolimus and tacrolimus levels showed seasonal variability highly consistent with changes in vitamin D; i.e. significantly lower drug concentrations in July–September than in January–March. As expected, no significant difference was evident for mycophenolic acid but this was
also the case with cyclosporine, possibly due to cross-reactivity of CYP3A4-mediated metabolites with the immunoassay used for quantification.

**Conclusion:** There is cyclic variation in blood levels of important immunosuppressants over the year that correlates with UV-light dependent changes in vitamin D levels. Even though a causal relationship remains to be established, it is suggested that individual differences in vitamin D may contribute to variability in drug metabolism and disposition.

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**P304**  
DOES GINKGO BILOBA EXTRACT EGB 761® INTERACT WITH SUBSTANCES METABOLIZED BY THE CYTOCHROME P450 SYSTEM? A RANDOMIZED, THREE-PERIOD, CROSS-OVER TRIAL

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**Introduction:** *In vitro* and animal studies led to the question whether extracts of Ginkgo biloba might have a potential to interact with the pharmacokinetics of substances metabolized by cytochrome P450 (CYP) enzymes, whereas available studies in humans did not support this.

**Patients and Methods:** A randomized, three-period, cross-over phenotyping trial was performed to assess the interaction potential of Ginkgo biloba extract Egb 761® with respect to the following CYP enzymes: CYP1A2 (as quantified using caffeine 3-demethylation), CYP2C9 (tolbutamide hydroxylation), CYP2C19 (omeprazole 5-hydroxylation), CYP2D6 (dextromethorphan demethylation), and CYP3A4 (midazolam hydroxylation). Eighteen healthy subjects, males and females, 18–55 years of age, were enrolled and completed three 8-day treatment periods (A, B, C) in random order, separated by 14-day washout periods. During each treatment period, subjects took one tablet in the morning and one tablet in the evening: placebo twice daily during period A, Egb 761® 120 mg twice daily during period B, Egb 761® 240 mg in the morning and placebo in the evening during period C. The morning dose on the eighth day was given together with oral administration of the cocktail of probe substances. Absence of an interaction was assumed if the 90% confidence interval for the estimated treatment/placebo ratio for a phenotyping metric was within the 0.70–1.43 range.

**Results:** Treatment/reference ratios of phenotyping metrics were close to unity for all CYP enzymes. Moreover, confidence intervals were within specified margins for CYP1A2, CYP2C9, CYP2D6, and CYP3A4 under 120 mg twice daily, as well as for CYP1A2, CYP2C9, CYP2C19, and CYP3A4 under 240 mg once daily. There were no conspicuous differences in rates of adverse events during active treatment and placebo or wash-out periods.

**Conclusion:** Egb 761® was safe and well tolerated. Altogether, clinically relevant effects on the activity of the CYP enzymes tested could be ruled out for both treatment regimens.

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**Table 1.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>0.3 mg i.m.</th>
<th>4 mg inhal.</th>
<th>8 mg inhal.</th>
<th>placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC0–t;max [ng*l/h]</td>
<td>204 (66.6)</td>
<td>236 (129)</td>
<td>282 (43.2)</td>
<td>81.6 (58.5)</td>
</tr>
<tr>
<td>AUC0–1.5 [ng*l/h]</td>
<td>130 (78.9)</td>
<td>148 (157)</td>
<td>215 (43.6)</td>
<td>40.0 (100)</td>
</tr>
<tr>
<td>Cmax [ng/l]</td>
<td>484 (162)</td>
<td>513 (220)</td>
<td>769 (78.9)</td>
<td>252 (559)</td>
</tr>
<tr>
<td>tmax [h]</td>
<td>0.22 ± 0.25</td>
<td>0.29 ± 0.31</td>
<td>0.21 ± 0.13</td>
<td>0.11 ± 0.11</td>
</tr>
<tr>
<td>Emax; Cmax [bpm*h]</td>
<td>78.0 (10.8)</td>
<td>88.2 (8.43)</td>
<td>89.2 (10.9)</td>
<td>87.4 (23.0)</td>
</tr>
<tr>
<td>AUEC0–1 h [bpm*h]</td>
<td>65.1 (8.22)</td>
<td>70.8 (10.4)</td>
<td>74.2 (7.45)</td>
<td>64.7 (6.29)</td>
</tr>
</tbody>
</table>

*Arithmetic means and standard deviation (SD); *significantly superior to i.m. administration, i.e. 90% CI for the ratios does not include unity.

**Conclusion:** This primarily exploratory study shows that administration of 4 or 8 mg epinephrine via inhalation provide at least equal and probably higher systemic exposure and more pronounced pertinent haemodynamic effects with similar tolerability profile compared to 0.3 mg epinephrine as i.m. injection. These data suggest that inhalative administration provides a higher chance of therapeutic efficacy in ambulatory emergency treatment of systemic hypersensitivity reactions. Mean exposure to epinephrine as derived from epinephrine plasma concentrations was highest for 8 mg inhal. and decreased over 4 mg inhal. to 0.3 mg i.m., with active treatments being significantly higher than placebo. Pronounced overall variability prohibited a definite assessment of relative bioavailability between treatments. However, 8 mg inhal. was not relevantly inferior 0.3 mg i.m. Significantly higher heart rates for the inhalative administrations compared to 0.3 mg i.m. were not accompanied by excessive changes.

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**P307**  
A TOTAL GASTRECTOMY INCREASES HEPATIC CYTOCHROME P450 EXPRESSION BY INCREASING LITHOCHOLIC ACID-PRODUCING INTESTINAL FLORA IN MICE

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**Introduction:** It has been found that partial gastrectomy patients showed decreased AUC (area under the blood concentration-time curve) of oral treatment with imatinib and propranolol. Although an increase in the gastric emptying rate (GER) is considered the primary cause for this decrease, the real mechanism has yet to be fully understood. This study examined the effect of gastrectomy on the expression of the hepatic drug metabolizing enzyme, Cytochrome P450 (CYP).

**Materials and Methods:** The mRNA expression level of hepatic Cyp3a11 was measured for the mice at 2, 4, 12, and 24 weeks after a total gastrectomy. The protein expression level and metabolic activity of Cyp3a in hepatic microsomal fraction were examined. The contents of
lithocholic acid (LCA)-producing bacteria (Bacteroides fragilis) and LCA in the feces were measured.

**Results:** At 12 and 24 weeks after the gastrectomy, the mRNA expression level of Cyp3a11, the protein expression level and metabolic activity of Cyp3a in the liver, along with the contents of Bacteroides fragilis and LCA in the feces significantly increased compared to the sham operation group. These changes were not observed at 2 and 4 weeks after the gastrectomy.

**Conclusion:** These results indicate the possibility that an increase in the expression level and activity of hepatic Cyp3a along with the increase in GER, contributed to the decrease in AUC of imatinib and propranolol in gastrectomy patients. It was revealed that the gastrectomy caused the increase in LCA-producing enterobacteria, and the increased content of LCA caused the increase in the hepatic Cyp3a expression level.

**P309**

**EFFECTS OF ITRACONAZOLE, RIFAMPICIN AND GRAPEFRUIT JUICE ON THE PHARMACOKINETICS OF NADOLOL IN HEALTHY VOLUNTEERS**

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**Aims:** Our objective was to evaluate the effects of itraconazole, rifampicin and grapefruit juice on the pharmacokinetics of the hydrophilic β-adrenoceptor blocker nadolol in healthy volunteers.

**Methods:** Twelve healthy male volunteers were studied by use of a randomised, open-label, 4-way crossover design with 14-day washout. A single oral dose of 30 mg nadolol was administered with water (control), 100 mg of itraconazole or 300 ml of grapefruit juice, or after a 6-day pretreatment with rifampicin (450 mg/day). The plasma concentrations and the urinary excretions of nadolol were measured up to 48 h after its dosing with high-performance liquid chromatography. Systolic and diastolic blood pressures and heart rate were recorded in a sitting position before and 4 h after the administration of nadolol and 0.5, 1, 2, 3, 4, 6, 8, 10, 24 and 48 h later. Pharmacokinetic analysis was performed using WinNonlin software.

**Results:** Itraconazole increased the peak plasma concentration and the mean area under the plasma concentration-time curve (AUC0-48) of nadolol 3.3-fold and 2.1-fold, respectively (P < 0.05). A slight, but not statistically significant, decrease in AUC0-48 of nadolol was observed in rifampicin and grapefruit juice phases as compared to the control. The elimination half-life for nadolol did not differ significantly among the four phases. During itraconazole phase, nadolol reduced systolic and diastolic blood pressures and heart rate greater than the other phases.

**Conclusion:** These results suggest that itraconazole substantially increases the bioavailability of nadolol, whereas rifampicin pretreatment and grapefruit juice slightly decrease the plasma concentration of nadolol.

**P309**

**THE INFLUENCE OF METHYL ESTER OF MONOKETOCHELIC ACID ON QUININE ENTERING THE CENTRAL NERVOUS SYSTEM IN RATS**

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**Introduction:** The study analyzes the influence of newly synthesized methyl ester of monoketocholic acid (MKH ME) on quinine entering into CNS.

**Methods:** The experiment was carried out on anesthetized white rats (n = 48), which had quinine injected by retrograde intraarteral bolus injection (15 s) into their right axillary artery. With such an application, quinine was directed toward brachiocephalic trunk, and with blood circulation reached head i.e. CNS, thus avoiding the influence of peripheral organs which could change its resorption, distribution, metabolism and excretion. The animals in control and test groups received subcutaneously equimolar doses of physiological solution and MKH ME 30 min before intraarterial application of quinine. The rats (minimum six animals) were decapitated 30, 60, 150 and 240 s after quinine application. Blood samples were taken from the left jugular vein, and the brain was, after being rinsing out, divided into cerebral trunk, cerebellum, and cerebral hemispheres for determining of quinine concentration by standard spectrophotofluorimetric methods.

**Results:** Quinine reached maximum concentrations in CNS latently in comparison with the maximum concentration in blood, and they were mainly higher in CNS than in blood. Quinine central kinetics showed the existence of the two compartments in CNS, one, consisting of the cerebral trunk and cerebellum, and where the concentrations are higher; and the other consisting of cerebral hemispheres.

**Conclusion:** Methly ester of monoketocholic acid made quinine entering the CNS significantly easier. The study was supported by grant from project No 41012, Ministry of Science, Republic of Serbia

**P310**

**INTERFERENCE OF BILE ACID DERIVATIVES WITH MORPHINE ANALGESIC EFFECT**

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**Introduction:** Bile acids and their salts can be used for improvement of xenobiotics absorption. Bile acid salts improve drug permeability through blood-brain barrier. There is also data on improvement of nasal, intestinal, buccal, transdermal, ocular, rectal and pulmonary absorption.

**Methods:** Influence of sodium salt of monoketocholic acid MKH-Na and methyl ester of monoketocholic acid MKH-Me on morphine (2 mg/kg intramuscular administration) analgesic effect in mice was examined. Antinociceptive hot plate method was used to estimate analgesic effect of morphine. Interaction was estimated by detection of changes in analgesic effect of morphine when combined with bile acids (subcutaneously administrated, dose of 4 mg/kg 20 min prior to morphine administration) compared to analgesic effect of the same dose of morphine given alone. Analgesic effect was measured for 90 min (in 10 min intervals) from morphine administration.

**Results:** Liposoluble methyl ester of monoketocholic acid did not show interference with morphine analgesic effect, what was in contrary to our expectations since morphine is a liposoluble drug. However hidrosoluble sodium salt of monoketocholic acid increased the analgesic effect of morphine in first 20 min from morphine administration.

**Conclusions:** According to the time point when interaction reached statistically significant difference it can be presumed that after intramuscular administration of morphine, sodium salt of monoketocholic acid increases morphine absorption and transport to brain and in that way increasing morphine analgesic effect.

The study was supported by grant from project No 41012, Ministry of Science, Republic of Serbia.
RATIONAL DRUG PRESCRIBING

P312
MULTIPLE DRUG THERAPY IN INTERNAL MEDICINE WARDS
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Introduction: Multiple drug therapy is an often observed fact among hospitalized patients, especially elderly. In aim to improve the quality of the pharmacotherapy the polypharmacy needs to be recognized and drug associations used only when rational and optimal.

Methods: In this retrospective study we have analyzed case report forms from 303 adult, hospitalized patients. The most important inclusion criterion was the presence of a cardiovascular diagnosis, while exclusion criteria consisted of oncological, end stage disease patients. We have evaluated the confirmed diagnoses and the administered drug therapy in concordance with the current guidelines. The use of more than five medicines concomitantly was considered polypharmacy.

Results: Our study revealed that using an amount of drugs is frequent: 215 from 303 patients received more than five drugs simultaneously, moreover 34 from these were administered more than ten drugs at the same time. The most often diagnoses were arterial hypertension 57.09%, chronic heart failure 55.77% and ischemic heart disease 54.45%. Regarding pharmacological therapy we observed the very frequent use of ACE inhibitors, different – not potassium sparing diuretics and certain gastrointestinal medicines, especially H2-blockers and antacids. As a final summarizing of our analysis, we can say, that polypharmacy is very common in the studied population.

Conclusions: The purpose of this study was to determine the frequency of multiple drug therapy and to recognize further possibilities of improving treatment. We conclude that the high amount of medicines given concomitantly can cause hidden adverse events and also the prolongation of hospitalization. Therefore a widened analysis of pharmacotherapy is planned, and quality improving measures should be implemented.

P313
‘WHOSE JOB IS IT ANYWAY’ – SWEDISH GENERAL PRACTITIONERS’ PERCEPTION OF THEIR RESPONSIBILITY FOR THE PATIENT’S DRUG LIST
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Introduction: Information about the patient’s current drug list is a prerequisite for safe drug prescribing. The aim of this study was to explore general practitioners’ (GP’s) understandings of who carries the overall responsibility for the patient’s drug list so that drugs prescribed by different physicians don’t interact negatively or even cause harm. The study also sought to clarify how this responsibility was managed.

Methods: A descriptive qualitative study was conducted among 20 Swedish physicians. The informants were recruited purposively and their view on responsibility was captured by semi-structured interviews. Data were analyzed using a phenomenographic approach.

Results: This study shows a variation in understandings about who is responsible for the patient’s drug list and in particular how the GP’s use different strategies to manage this responsibility. This is described in five categories: (i) Imposed responsibility, (ii) Responsible for own prescriptions, (iii) Responsible for all drugs, (iv) Different but shared responsibility, and (v) Patient transferring drug information. The relation between categories is illustrated in an outcome space which describes how the GP’s reason in relation to managing drug lists.

Conclusions: The understanding of the extent of GP responsibility for the patient’s drug list varied, which may be a threat to safe patient care. We propose that GP’s are made aware of the variations in understandings in order to improve health care quality.

P314
A PILOT RANDOMIZED CONTROLLED TRIAL OF DEPREScribing: STOPAT (SYSTEMATIC TERMINATION OF PHARMACEUTICAL AGENTS TRIAL)
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Introduction: Polypharmacy and adverse drug reactions are frequent and important among older people. We have found substantial evidence of potentially suboptimal use of medications among older people, (i) especially those living in residential care facilities. (ii) Few clinical trials have evaluated systematic withdrawal of medications among older people. This small, open, study was conducted to determine the feasibility of a randomised controlled deprescribing trial.

Methods: Ten volunteers living in the community (recruited by media advertising) and 25 volunteers living in residential aged-care facilities (RCF) were randomised to intervention or control groups. The intervention was gradual withdrawal of one target medication. The primary outcome was the number of intervention participants in whom medication withdrawal could be achieved. Other outcomes measures were quality of life, medication adherence, sleep quality, and cognitive impairment.

Results: Participants were aged 80 + 11 years and were taking 9 + 2 medications. Fifteen participants commenced medication withdrawal and all ceased or reduced the dose of their target medication. Two subjects withdrew; one was referred for clinical review, and one participant declined further dose reductions.

Conclusion: A randomised controlled trial of deprescribing was acceptable to volunteer participants. Recruitment in RCF is feasible. We are now initiating further deprescribing trials in residential care populations, as a prelude to definitive trials of deprescribing.

References:
1. Beer et al, BJCP, 2011 In press

This study was supported by a Royal Perth Hospital Medical Research Foundation grant.

P315
POTENTIAL DRUG-DRUG INTERACTIONS IN OUTPATIENT PRESCRIPTIONS IN A GENERAL HOSPITAL
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Introduction: Adverse drug interactions increase morbidity and mortality. To prevent this we analysed prescriptions in outpatients in our hospital.

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Method: Prescriptions administered to outpatients during 1 month (November 2009) were analysed to identify potential DDIs appearing on the same prescription sheet. The MEDSCAPE data base was used for the study. The program uses two severity rating scales simultaneously to determine the level of severity of a particular drug-drug interaction. The severity of interaction is determined by the description of the measure as mild/moderate/severe/no interaction and in the form of numbers (classes) ranging from 1 to 5 determining their level of severity in terms of clinical significance.

Results: The number of prescriptions with IDDs for men were 45.45% and for women 54.54%. The most common diseases in the prescription with IDDs were: coronary disease 41.66%, depression 25%, epilepsy 16.66%.

Combinations/interactions found:
1. Clopidogrel/atorvastatin: 18.18%. Class 3 moderate interaction, decreased in clopidogrel-mediated platelet aggregation inhibition.
2. Clopidogrel/proton pump inhibitors: 18.18%-2 severe interaction, decreased clopidogrel effectiveness.
3. Antidiabetics/beta blockers: 9.09% -3 moderate interaction, may occur with IDDs were: coronary disease 41.66%, depression 25%, epilepsy 16.66%.

Conclusion: The easiest way to reduce the frequency of IDDs is to decrease the number of medicines prescribed. Nevertheless sometimes it is difficult to reduce the number of drugs prescribed for patient with multiple chronic conditions. Therefore, it could be necessary to make a careful selection of therapeutic alternatives and in cases without other options, patients should be continuously monitored to indentify adverse events. Doctors always must know well the patients medical history, use the appropriate DDDIs programs by internet and take advise by pharmacologists.

P316 AN ASSESSMENT OF PRESCRIPTIONS WRITTEN FOR THE TREATMENT OF DYSPEPSIA
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Introduction: Dyspepsia is one of the most common diseases in the community. In this study, it was aimed to review the details of dyspepsia diagnosed prescriptions which were written out by physicians practicing at different institutional levels of health care.

Method: A total of 274 prescriptions by physicians practicing at primary health care centers (110 prescriptions at PHCCs) and hospitals (164 prescriptions at Hs) in 10 provinces of Turkey were collected in pharmacies and analyzed by the most frequently prescribed medicines, number of medicines per prescription (MPP), treatment cost per prescription (TCP) and the classifications in the recent pharmaceuticals list [Gold Standard(GS)] which was developed for dyspepsia treatment.

Result: MPP average was 1.44 ± 0.71 in PHCCs and 1.59 ± 0.88 in Hs. TCP average was 32.94 ± 22.46 TRL in PHCCs, 35.51 ± 40.06 in Hs. GS medicines were prescribed in 55.7% of the prescriptions written in PHCCs and 68.7% of the prescriptions written in Hs. Lansoprazole (22.7%), pantoprazole (12.7%), esomeprazole (6.7%), alginic acid (6.4%) and famotidine (4.1%) were the first 5 medicines prescribed.

Conclusion: The findings indicated that the physicians practicing at PHCCs had a better performance than the physicians practicing at Hs pertaining to the quantity and cost of the medicines. With regards to prescribing in compliance with the treatment guidelines for dyspepsia, however, the performance of the physicians practicing at PHCCs was found to fall behind that of the physicians practicing at Hs. These findings should be utilized in order to improve the actions to be taken for rational drug use in the future.

P317 PRESCRIBING PATTERN OF ANTIMICROBIAL AGENTS TO PATIENTS WITH CHEMOTHERAPY-INDUCED FEBRILE NEUTROPENIA AT SQU HOSPITAL

Background: Neutropenia is the most significant risk factor for infection in cancer patients treated with chemotherapy, especially hematological malignancies. Therefore, international guidelines recommend immediate initiation of empiric antibiotic therapy. There is no data on the incidence or management of febrile neutropenia (FN) in cancer patients treated by chemotherapy in Oman.

Objectives: 1 To describe the prescribing pattern of antimicrobials to patients with hematological malignancies who developed FN at SQUH. 2 To compare the actual use of antimicrobials in FN with local and standard guidelines. 3 To determine the type and frequency of culture isolates from the FN episodes.

Study Design and Setting: This is a retrospective observational study covering a period of 3 years (from January 2007 to February 2010). FN episodes were studied in patients with hematological malignancies (leukemias, lymphomas and myelomas) in three different wards (adult hematology, paediatric hematology and oncology unit) at Sultan Qaboos University Hospital. The Hospital Information System (HIS) program called ‘Trakcare’ was used to extract relevant patients’ information.

Main Results: A total of 107 febrile neutropenia episodes were analyzed. Sixty-four percent had two episodes during the analysis period. More than one third (35%) had severe neutropenia (ANC<100 cells/mm³). The duration of neutropenia was >1 week in the majority of the episodes (57%). FN developed during hospital stay in 57% of the episodes and lead to hospital admission in remaining 43%. The mean duration of treatment was approximately seven days with no significant difference between specialties or different types of malignancies. Only 34 (19%) episodes had positive cultures; mostly from blood (30 episodes; 88%). The majority of isolates were Gram negative organisms (63%). The initial empirical treatment included monotherapy (37%), dual therapy (60%) and triple therapy (3%). There was a significant variation in the choice of the initial empirical antimicrobial regimens between the three specialties managing FN episodes.

Conclusions and Recommendations: This study showed that there is a large variation in the antimicrobial treatment of FN episodes in patients with hematological malignancies at SQUH. All chosen drugs were within the international guidelines’ recommendations. However, due to nature of the study it was not possible to determine the incidence of FN, or the factors affecting the choice of its antimicrobial treatment. Since knowledge of the local microbial isolates and resistance patterns are very important in deciding the first choice of empirical antimicrobial therapy for FN, this retrospective analysis highlights the need to determine prospectively the factors that may influence the choice FN antimicrobial therapy including: the microbial profile, local antimicrobial sensitivity and resistance patterns so that the hospital can develop local practice guidelines based on local evidence. It is also important to determine the incidence and outcome of FN in patients treated at SQUH to provide optimal therapy.

P318 THE PRESCRIBING TRENDS IN OBSTRUCTIVE AIRWAY DISEASES IN OUTPATIENT SETTING OF MONTENEGRO 2000–2010: A LONGITUDINAL OBSERVATION
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Introduction: The aim of this study was to investigate the prescribing of drugs for obstructive airway diseases (OAD) in outpatient setting of Montenegro in 2000–2010, to evaluate the doctors’ adherence to actual
guidelines and to explore possible medical and non-medical factors that could influence the drug prescribing during investigated period. 

**Methods:** Data on drugs that were prescribed during 2000 within Primary health care (PHC) and reimbursed by the Republic Health Insurance Fund of Montenegro (RHFIM) were obtained from Wholesalers’ Annual Reports. At that time, it was the only possible source of information on drugs prescribed for the treatment of OAD (ATC code R03). Data on medication prescribed in 2010 were extracted from the National database that was set up within RHFIM in 2004. Our retrospective-prospective longitudinal study comprised a sample of 100% of drugs prescribed for OAD per year in outpatient setting of Montenegro. Entire population of Montenegro (approximately 660,000 inhabitants) was covered by this study. Internationally accepted ATC/DDD methodology was applied.

**Results:** The prescribing of drugs for OAD within PHC in Montenegro increased for approximately 150% in 2010 compared to 2000 (17.0 vs. 6.8 DDD/1000 inh./day). The structure of prescribed drugs was also different. In 2000, the most prescribed drugs were short-acting b2 agonist salbutamol (3.7 DDD/1000 inh./day, 54%), bronchodilatator aminophylline was on the second place (1.3 DDD/1000 inh./day, 19%) and beclomethasone was the third drug (0.9 DDD/1000 inh./day, 13%). In 2010, the prescribing of fixed combinations was significantly increased. Fenoterol/brompheniramine combination was prescribed mostly (5.8 DDD/1000 inh./day, 34%) and combination of long-acting b2 agonist salmeterol with inhaled corticosteroid flutikazon was on the second place (3.0 DDD/1000 inh./day, 18%). Aminophylline was prescribed at the rate of 2.3 DDD/1000 inh./day (14%).

**Conclusions:** Our results suggest that in the period 2000–2010 significantly increased the prescribing of drugs for OAD in Montenegro that have reduced the difference in the volume of prescribed drugs in relation to developed countries. Like in developed countries, the structure of drugs was also changed in favor of fixed combinations, which probably indicates an improvement in pharmacological approaches to OAD.

**Keywords:** drugs prescribing, obstructive airways diseases.

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**P319 PRESCRIPTION PATTERNS OF DISPENSING DOCTORS AND OTHER MEDICINE PROVIDERS**

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**Objective:** The main objective of this study was to analyse the prescribing patterns of dispensing doctors and other medicine providers in a section of the private health care sector of South Africa.

**Method:** A retrospective drug utilisation study was performed on medicine claims data of a pharmacy benefit management company in South Africa during 1 January 2005 until 31 December 2008. Dispensing doctors were classified as doctors who prescribed medicines and provided them. Other health care providers included non-dispensing doctors and specialists who only prescribed medicines (fulfilling the role of prescriber); and pharmacists who only dispensed medicines (fulfilling the role of provider) or provided OTC and claimed it through the patient’s medical scheme. [RSA Rand (R/SUS) = 6.38112 (2005); 6.78812 (2006); 7.06926 (2007) and 8.27505 (2008)].

**Results:** The results revealed that dispensing doctors had a lower cost per prescription compared to other health care providers (R112.44 vs. R256.77) and also had a lower cost per medicine item (R39.48 vs. R112.00) for the entire study period from 2005 to 2008. Dispensing doctors provided more items per prescription compared to other health care providers (2.85 items vs. 2.29 items) but other health care providers claimed more prescriptions per patient per year (7.30 prescriptions vs. 3.30 prescriptions). A higher percentage of generic medicine items were prescribed to patients compared to non-dispensing doctors. The results also revealed that dispensing doctors provided relatively inexpensive medicine items, including generic and innovator items, for female and male patients of all ages while other health care providers showed the opposite trend and issued relatively expensive medicine items to these patients.

**Conclusion:** From 2005 to 2008 dispensing doctors in South Africa issued more medicine items per prescription compared to other health care providers, but did so at a lower cost.

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**P320 DECISION SUPPORT SYSTEMS FOR NON-PRESCRIPTION DRUGS SELECTION**

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**Introduction:** There’s been a growing trend for people to self-medicate with non-prescription drugs for common diseases. Pharmacy personal have an important position in this matter, through their skills and knowledge, reducing and managing the risk and quality assurance. The objective of this paper is to perform a review research of scientific articles to realize the state of the art of the supply of non-prescription drugs using decision support systems.

**Methods:** To carry out the review, was performed a research for scientific articles on Pubmed and ISI Web of Knowledge.

**Results:** From all articles matching the imposed conditions, 21 were chosen to full text review. Retrieved articles address issues related to computer systems in community pharmacies (6), medicines adverse reactions and/or interactions communication and alert (5), supply of non-prescription drugs (4), medication errors (3), safety (2) and counselling (1).

**Conclusion:** On the supply of non-prescription drugs, a computer-based system will absolutely improve the efficiency of the Pharmacy Professional, reducing risk and errors, and increasing incident alerts, safety and ideal counselling. Studies and assessments undertaken on the subject will conduct to the promotion of safety and better professionalism.

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**P321 LONGITUDINAL ANALYSIS OF MEDICINE PRESCRIBING PATTERNS FOR METABOLIC SYNDROME PATIENTS IN SOUTH AFRICA**

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**Introduction:** Identifying individuals at risk for chronic diseases is the first step towards preventive measures. Metabolic syndrome is a diagnosis that has been proposed to identify patients in whom the clustering of risk factors is associated with increased risk of diabetes and cardiovascular disease. In the absence of data amongst South Africans, the aims of the study are to determine the prescribing patterns and trends for hypoglycemic, antihypertensive, and hipolipidemic agents using data from a South African Pharmaceutical Benefit Management company.

**Patients and Methods:** A non-experimental, quantitative retrospective drug utilisation was performed using prescription claims data for a 4-year period (January 1, 2005–December 31, 2008). Metabolic syndrome patients were defined according to the AHA/NHLBI criteria, as a those who had a prescription(s) for one or more drugs, from each of the following therapeutic drug classes: hypoglycemics, antihypertensives and hipolipidemics. A total of 14,914 patients from 2005 were included in the analysis, compared with 17,372 from 2006, 17,464 from 2006 and 17,866 from 2008.

**Results:** In 2005, patients received 191,747 prescriptions, which increased by 23.9% to 237,503 in 2006. A further 4.1% increase in this number was then observed to 247,322 in 2007, followed by a marginal 0.4% drop off to 246,279 prescriptions in 2008 (a relative increase of 28.4% from 2005 to 2008). The number of items dispensed increased by an overall 35.1% from 2005 to 2008, after an initial 25.2% increase to 24.2% (n = 616,359) in 2006, 26.3% (n = 663,052) in 2007, and 28.4% from 2005 to 2008). The number of items dispensed increased by 23.9% to 237,503 in 2006. A further 4.1% increase in this number was then observed to 247,322 in 2007, followed by a marginal 0.4% drop off to 246,279 prescriptions in 2008 (a relative increase of 28.4% from 2005 to 2008). The number of items dispensed increased by an overall 35.1% from 2005 to 2008, after an initial 25.2% increase to 24.2% (n = 616,359) in 2006, 26.3% (n = 663,052) in 2007, and 28.4% from 2005 to 2008).
Near-misses are a frequent occurrence in hospitals where clinical practice in this field.

**Method:** A paper-based survey was conducted on 162 final-year medical students attending a London Medical School in August 2009. The survey consisted of 23 items covering a broad range of prescribing issues with two questions focusing specifically on how likely individuals would be to report errors. Ethical approval was obtained in-house at Barts and the London School of Medicine and Dentistry.

**Results:** In response to the question: 'If you made a prescribing error that led to actual patient harm, would you report it?' 96.82% of the respondents said that they would report it. However, when asked: 'If you made a prescribing error that nearly led to patient harm, would you report it?' only 34.23% said that they would report it.

**Conclusion:** The most interesting yet concerning finding of this study was that almost two-thirds of respondents did not recognise the importance of reporting near-misses in order to improve medication safety.

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**P323**

**SURVEY OF DRUG PRESCRIBING HABITS OF CROATIAN INTERNAL MEDICINE RESIDENTS IN TWO MAJOR TERTIARY CARE HOSPITALS IN ZAGREB**

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**Introduction:** The aim of this survey was to assess the level of confidence of internal medicine residents concerning drug prescribing during their residency.

**Materials and Methods:** A questionnaire-based observational study was conducted in two major hospitals in Zagreb during February 2011. The participants were 33 internal medicine residents of different levels of experience. Main outcome measures were derived from demographic data and self-reported drug prescribing habits assessed by means of a 10 item long online questionnaire. The obtained data were used to describe the residents’ drug prescribing skills and attitudes towards rational pharmacotherapy.

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**P324**

**ASSESSMENT OF CONTROLLED MEDICINES PRESCRIPTION PATTERNS IN THE CONTEXT OF GREEN AND RED COLOURED SCRIPTS**

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**Introduction:** In Turkey, controlled ‘psychotropic drugs’ and ‘narcotic drugs’ are allowed to use in the therapy under the condition if they are written out on ‘green coloured scripts (GCS)’ and ‘red coloured scripts (RCS)’ respectively. In this study, it was aimed to reveal controlled medicines (CM) utilization by comparing content of GCS and RCS.

**Method:** A total of 52000 GCS and RCS (4000 prescriptions/month) were collected retrospectively (December 2008–December 2009) from Provincial Health Directorate of Istanbul. GCS and RCS were analyzed by patients’ gender, indications, physicians’ specialties and CM-related details.

**Results:** The majority of scripts were green coloured (78.6%). GCS were mostly prescribed by physicians from psychiatry (37.4%) while RCS were mostly prescribed by physicians from child psychiatry (35.0%). The most frequently prescribed CM on GCS was alprazolam (39.4%) and on RCS was methylfenidate (51.1%). The most commonly written indication on GCS was anxiety (30.8%) whereas RCS was ‘attention deficiency-hyperactivity disorders’ (53.5%). GCS was prescribed mostly for women (55.4%) whereas RCS was prescribed mostly for men (67.8%; P < 0.001). Prescription content was found to be similar for each month.

**Conclusion:** The findings indicated that there was a tendency towards use of controlled psychotropic medicines mostly by women while narcotics mostly by men. Frequently prescribed CM were prescribed by the psychiatrists. There was no seasonal difference among the prescribed CM in each month. All these findings may serve as a guide for the development of CM follow-up systems and regulations.
THE WAY STORING AND ANALYSIS OF INFORMATION ABOUT DRUG-PRESCRIBING

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Introduction: The threat of the microbial resistance to the antibiotics (AB) causes the clinicians to look for the best way storing and analysis of information about AB-prescribing as well as the characteristics of the microbial resistance. The monitoring of the AB-prescribing and microbial resistance is a very important part of the Antimicrobial Stewardship Policy all over the world. That is a very important reason for organizing the International Electronic Data-bases.

Materials: The decisions for AB-prescribing depend on the etiology, severity, stage, phase of the definite infectious disease or definite situation and they should be systematized. The principles of ‘quantization’ and systematization of the Informative Continuum of Clinical Events were created and tested.

Results: The implementation of the Protocols for the restricted AB-prescribing, as defined in the structure of the five Classes of Clinical Events (5-qu-bits segmentation of information), brought an improvement in the quality of Clinical Practice and Clinical Results and gave a substantial saving (50%) in the lives of the adult patients with severe pneumonia.

Conclusions: The first experience of modeling the Patterns for decision making AB-prescribing on the base of principle of ‘quantization’ of the Clinical Informative Continuum by using 5-qu-bits segmentation of information, showed its applicability for creating the protocols, modeling the drugs expenditure plans, and monitoring the quality of Clinical Practice. This methodology may be useful in the future for getting the new knowledge for the Clinical Practice.
PSYCHIATRIC DISORDERS

P326
ENHANCEMENT OF DOPAMINERGIC BRAIN FUNCTIONS BY EXERCISE
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Introduction: We previously suggested that calcium increases dopamine synthesis in the brain through a calmodulin-dependent system. On the basis of this mechanism, the effect of exercise on brain function was investigated through animal experiments.

Methods and Results: Effect of exercise on hypertension: Male spontaneously hypertensive rats (SHR) were forced to run at a speed of 10 m/min using a programmed motor-driven wheel cage. It was shown that exercise leads to increased serum calcium levels, and the calcium is transported to the brain and in turn enhances brain dopamine synthesis. The decrease in serum calcium levels, which might be due to a decrease in the availability of bone calcium, causes a decrease in dopamine synthesis in the brain and hypertension in SHR. In particular, there were abnormally low levels of dopamine in the neostriatum and nucleus accumbens regions, which were improved following intracerebroventricular administration of calcium chloride. Moreover, the decrease in dopamine synthesis and hypertension in SHR were normalized by exercise. Role of convulsions in epilepsy: The decrease in calcium-dependent dopamine synthesis was also found in epileptic mice (EL mice strain). Their own convulsions normalized brain dopamine levels and physiologic functions in EL mice, and the effect was very similar to that of administration of calcium.

Conclusions: These findings suggest that exercise enhances dopamine synthesis in the brain, and subsequently increases dopamine regulates various brain functions. This mechanism might underlie the rectifying effect of exercise on hypertension, and the rectifying effect of convulsions on brain function disorders in epilepsy. This mechanism might also underlie the ameliorative effect of exercise on the symptoms in Parkinson’s disease or senile dementia, because dopaminergic function is abnormally reduced in these diseases.

References:

P327
NEUropsychiatric MANIFESTATIONS IN DOGInox INTOXICATION
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Introduction: The aim of the study was to determine the incidence of neuropsychiatric changes in a group of patients hospitalized with pathological plasma digoxin levels and to correlate these changes with other manifestations of digitalis intoxication.

Patients and Methods: We analyzed clinical and laboratory data including drug therapy data obtained from the medical records of patients hospitalized over the last 36 months.

Results: Plasma digoxin levels above 2 ng/ml were seen in 171 patients, clinical manifestations of intoxication were demonstrated in 106 patients, and present in all patients with plasma levels above 3 ng/ml. Neuropsychiatric manifestations were noted in 15% of all patients.

Conclusions: Neuropsychiatric manifestations are not specific, and they may be difficult to diagnose, especially in elderly patients. Their lower incidence in our group of patients is due to the retrospective nature of our analysis. Risk factors of intoxication included very old age and renal dysfunction. Because of the concomitant use of drugs, drug interactions may have played a significant role in the elevated digoxin levels.

P329
ASSOCIATION OF POLYMORPHISMS IN THE DOPAMINE AND SEROTONIN GENE SYSTEMS WITH RISK FOR ANOR-EXIA Nervosa AND PSYCHOPATHOLOGICAL TRAITS
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Introduction: The serotonergic and dopaminergic systems are involved in the regulation of mood, and social and eating behaviour. Therefore, polymorphisms in genes participating in these pathways hold the potential to play a role in the pathogenesis of eating disorders such as Anorexia Nervosa (AN).

Methods: We evaluated the presence of common polymorphisms in the serotonin receptor 2A gene (5HT2AA-1438G), the serotonin and dopamine transporter genes (5HTTLPR L/S and SLC6A3 40bp VNTR) and the catechol-orthomethyl transferase gene (COMT V158M) in 120 AN patients and 212 healthy subjects. Psychopathological traits were assessed by EDI-2 and SCL-90R inventories.

Results: The 5HTTLPR-S (short) allele and the -1438G variant were significantly associated to longer disease evolution (P = 0.02 and P < 0.01, respectively). None of the polymorphisms analyzed were individually associated with the risk for AN. However, a statistical trend was observed towards lower risk for AN in subjects who were carriers of at least one mutant homozygous genotype (5HTTLPR S/S and/or 5HT2AA -1438GG). These individuals accounted for 45.8% of controls but only 36.7% of cases [OR = 0.68 (0.40–1.10), P = 0.06].

The study of personality dimensions revealed that the 5HT2A-1438GG genotype was associated to higher scores in the Positive Symptom Total subscale of the SCL-90R questionnaire (P = 0.035). Moreover, carriers of the 5HT2A-1438GG genotype and at least one 5HTTLPR L allele scored higher in 11 out of the 15 categories assessed. Differences in Bulimia and Interoceptive Awareness (EDI-2) reached statistical significance (P < 0.05).

Finally, the nine-repeat allele in the SLC6A3 gene was associated with increased scores (P = 0.04) in the Maturity Fear subscale of the EDI-2 test.

Conclusions: Our results suggest that polymorphisms in the serotonin and dopamine gene systems may interact to modulate the risk for AN and impact the clinical evolution of the disease.

Funding Support: This work has been supported in part by grants PR108A008, GR10122 and GR10022 from Junta de Extremadura, Consejería de Economia, Comercio e Innovacion, Merida (Spain), and grant PI-071152 from Plan Nacional de Investigación Científica, Desarrollo e Innovación Tecnológica (I+D+I), Instituto de Salud Carlos III, Madrid (Spain).

P330
MUSIC-DEPENDENT CHANGES IN DOPAMINerGic BRAIN FUNCTIONS
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Introduction: Previous our studies indicated that calcium increases dopamine synthesis in the brain through a calmodulin-dependent system, and that increased dopamine levels reduce blood pressure and prolong ethanol-induced sleep time (loss of righting reflex, unconsciousness). In this study, the effect of music on brain function was examined on this pathway.

Methods and Results: Measurement of blood pressure: Male spontaneously hypertensive rats (SHR) were placed in a closed cage equipped with a speaker, and music (Mozart, Adagio from Divertimento, K. 205;
average sound level = 65 dB) was played repeatedly. During and after music exposure, changes in blood pressure were monitored. Systolic blood pressure in SHR was reduced by exposure to music. The effect of music was abolished following inhibition of the calcium-dependent dopamine-synthesizing pathway in the brain. Exposure to music also increased serum calcium and neostriatal dopamine levels. Next, music was filtered to each frequency domain using the equalizer function, and changes in blood pressure were monitored. The blood pressure-reducing response was dependent on the frequency, and was markedly greater at 4–16 kHz compared with lower frequencies. Measurement of ethanol-induced behavior: The effect of music on brain function was reconfirmed in ddY mice (normal animals) through ethanol (4.5 g/kg)-induced sleep time. The ethanol-induced sleep time was prolonged by exposure to music, which was abolished by inhibition of the dopamine-synthesizing pathway.

Conclusions: These results suggest that music leads to increased calcium-dependent dopamine synthesis in the brain, thus causing reduction in blood pressure and enhancement in alcohol's effect. Especially high-frequency sounds stimulate dopamine synthesis, and might therefore regulate and/or affect various brain functions, leading to the amelioration of symptoms of various diseases that involve dopamine dysfunction.

Acknowledgements: This study was supported by a grant from the Yamaha Music Foundation, and by Grants-in-Aid for Scientific Research from the Japanese Society for the Promotion of Science (17927001, 18927001).

References:

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REPEATED ADMINISTRATION OF CAFFEINE INDUCES COCAINE- AND AMPHETAMINE-TRANSCRIPT REGULATOR (CART) OVER-EXPRESSION IN MICE: COMPARISONS WITH COCAINE

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Cocaine- and amphetamine-transcript regulator (CART) can be induced by cocaine and amphetamine with correlation to the behavioral sensitization during the development of reward and dependence. These experiments were performed to know whether caffeine induces CART, and it's signaling, compared with those of cocaine.

Behavioral sensitization was developed in mice during 7 days of repeated treatments with caffeine. Subsequently, altered CART sequences, levels of CART mRNA and peptides in dose and time responses to treatments in striatum and it's cAMP/PKA/pCREB signaling at 5th day of treatments were assessed and also compared with those of cocaine.

Behavioral sensitization by cocaine was induced on set of days 2 and on process of 6 days, whereas caffeine induced behavioral sensitization limit on days 5. Subsequently alignment of sequences of CART with treatments was completely homology. As the protein recording from striatum of treatments, levels of CART peptide were peaked on day 5 with no corresponding difference in levels of CART mRNA. Within striatum, significantly largest numbers of CART+ cells by cocaine were seen within nucleus accumbens (NAcc) shell whereas cocaine increased greater numbers of CART+ cells within dorsolateral (DL) striatum compared with NAcc shell. Furthermore, antagonists of D1 and D2 receptors blocked over-expressions of CART by cocaine via inhibiting the cAMP/PKA/pCREB signaling. However, antagonists had no effects that by caffeine.

Taken together, repeated caffeine induces CART, and striatal CART over-expression induced by caffeine would potentially modulate drug reward systems.

Keywords: striatum; CART; drug rewards; dopamine pathway; cAMP/PKA/pCREB signaling.
RARE DISEASE AND ORPHAN DRUGS

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EAST ASIAN ETHNICITY AND DRUG DEVELOPMENT: IMPLICATIONS FOR ELMTROMBOGAP IN CHRONIC IDIOPATHIC THROMBOCYTOPENIC PURPURA (CITP).

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Inter-ethnic differences in response have been observed for a range of drugs although few are dosage according to patient ethnicity. Eltombogap is a nonpeptide thrombopoietin receptor agonist approved for previously-treated patients with CITP. The initial recommended dose (50 mg/day for most cITP patients) is subsequently individualised based on platelet response. Across the multinational clinical development program target platelet counts (50–400 × 10⁹/l) were achieved at lower median average daily doses in East Asian than White subjects (e.g. TRA102537 52.9 mg/day vs. 64.6 mg/day). Additionally a slightly greater proportion of East Asian than White subjects experienced supratherapeutic responses (platelets >400 × 10⁹/l). When dosing was adjusted to achieve target platelet counts a clinically important inter-ethnic difference in bleeding was not observed. A trend to a higher incidence of hepatobiliary adverse events in East Asian than White cITP subjects was observed although the overall incidence of adverse events was similar in East Asian and White cITP subjects. Pharmacokinetic studies and population analyses indicated that eltombogap exposure was higher in both healthy and cITP subjects of East Asian than non-Asian ancestry (50 mg/day: 87% higher AUC0-tau in East Asian cITP subjects). The relationship between systemic eltombogap exposure and platelet response was similar in both ethnic groups. Therefore higher eltombogap exposures at the same dose in East Asian than White cITP subjects result in greater platelet responses in East Asian relative to White cITP subjects. Consequently eltombogap is dosed according to patient ethnicity with a lower initial dose (12.5 or 25 mg/day) recommended for cITP patients of East Asian ancestry (Japanese, Korean, Chinese).

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THE WORTHINESS OF MEASUREMENT OF CIRCULATING KISSPEPTIN LEVELS IN HYPOGONADISM PATIENTS

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Introduction: Kisspeptin, the product of the Kiss1 gene is a G-protein coupled receptor ligand for GPR54. It is recently become clear that kisspeptin-GPR54 signaling has an important role in initiating Gonadotropin releasing hormone (GnRH) secretion at puberty, the extent of which is under hormonal control by negative feedback from estrogen. Kisspeptin is there in circulating blood, and stably measurable by our enzyme immunoassay. Plasma kisspeptin levels represented low value at all times.

Conclusion: Kisspeptin is there in circulating blood, and stably measurable by our enzyme immunoassay. Plasma kisspeptin levels are expected to a good biomarker.

Rational Drug Prescribing

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SHORT BOWEL SYNDROME: CASE REPORT

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Introduction: Acquired short-bowel syndrome (SBS) is the malabsorptive state that follows extensive resection of the small intestine and it is associated with significant mortality and morbidity.

Patient and Method: 77-year-old man with extensive bowel resection with jejunostomy because of multiple hernias that left him dependent on total parenteral nutrition (TPN). After surgery, different schemes were tested: administration of enteral nutrition with pump to prevent osmotic load, with/without nasogastric tube or different formulas, all having high jejunal uptake. Finally, after a month of ileostomy we presented hyponatremia, so nutritional support was maintained by TPN supplemented with high amounts of sodium (177 mEq / bag) and insulin. The treatment included loperamide and codeine to slow gastric and intestinal transit, proton pump inhibitor (omeprazole) to suppress hypersecretion and vitamin supplements. PN was supplemented with an oral diet consisting of nutritional supplements (liquid, low osmolarity and without fiber) and regular food (physiological and psychological benefits). The patient was able to progress to cyclic PN, with an infusion of 8h. After discharge, he comes 3 days a week to receive PN.

Results: The nutritional absorptive capacity and digestive motility was studied for 12 months postoperatively. His absorptive capacity didn’t allow him oral nutritive autonomy and normal social life. Laboratory test showed a satisfactory nutritional profile but the patient has experienced several episodes of infection and poor quality of life.

Conclusions: Advances in long-term venous access, PN and changes in long-term management of chronic complications have altered the prognosis of patients with SBS.

Acknowledgements: None.

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DEPRESCRIBING IN FRAIL OLDER PEOPLE: PROTOCOL FOR A RANDOMISED CONTROLLED TRIAL

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Introduction: Polypharmacy causes significant morbidity and mortality in frail older people, but quality data on deprescribing are scarce. Our aims are to safely reduce the total number of medications taken by older people living in residential aged care facilities (RACF) and to estimate the effect of deprescribing on survival, falls and fractures, cognitive function, sleep quality, and independence.

Methods: We will randomly assign 250 volunteers aged ≥65 years to intervention and control groups. We will identify target drugs for withdrawal and sequentially withdraw as many as possible in intervention group participants. Participants will be closely monitored for adverse drug withdrawal effects and symptom recurrence. We will record the
medications taken and administer the Mini-Mental State Examination (MMSE), Neuropsychiatric Inventory–Nursing Home version (NPI-NH), and modified Barthel index at baseline and at 3, 6, and 12 months post-enrolment. We will record all deaths, falls, and non-vertebral fractures.

**Results:** Results will be analysed on an intention-to-treat basis. Our primary outcome will be the number of medications taken at 12 months post-enrolment. Secondary outcomes will be survival time, and number of falls and fractures at 12 months, and cognitive function (MMSE), sleep quality (NPI-NH) and independence (Barthel index) at 3, 6, and 12 months post-enrolment.

**Conclusion:** This study will be the first randomised controlled trial to investigate deprescribing. If we find that medication burden can be safely reduced in frail older people, our data will inform a large, multi-centre, deprescribing trial powered to detect clinically important effects on morbidity and mortality.

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**HOW TO IMPROVE THE USE OF PROTON PUMP INHIBITORS**

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**Introduction:** Proton pump inhibitors (PPI) are one of the most prescribed medications in the world with proven efficacy. However, several studies showed that their use often doesn’t respect indications, leading to over-consumption, thus exposing patients to drug interactions and adverse events (for example pneumonias). Interruption of PPIs can induce a rebound phenomenon. This generates costs for health systems.

**Methods:** This is a prospective interventional study performed in two hospitals: La Chaux-de-Fonds (CDF, cases) and Neuchâtel (NE, control) during two six-month periods, comparing use of PPIs before and after intervention. We elaborated recommendations (PPI doses and treatment duration) based on recent medical literature that we summarized on A6 cards and gave out to all prescribing doctors in the hospital of CDF and held a 30-minute information session for the departments of surgery, medicine and anesthesiology in March 2010. Doctors were asked to apply our recommendations as often as possible, leaving space for their own assessment. No information was given to the doctors of the control hospital. The number of PPI tablets that the pharmacy sent to each care-unit in both hospitals was counted and adjusted to the number of patient-days from April to September 2009 (before intervention) and April to September 2010 (after intervention). The number of other antacids that were used in both hospitals was counted during the same periods. General practitioners (GP) in the region around CDF received an explanation letter to avoid re-introduction, after discharge from the hospital, of PPI treatment stopped during the stay. The number of gastro-duodenal ulcers and upper digestive hemorrhages was counted from April to December 2009 and the same period in 2010 in both hospitals.

**Results:** In 2010, in the hospital of CDF, the use of PPIs per 100 patient-days decreased by 36% in the surgical and medical departments compared to 2009. In the control hospital the use of PPIs per 100 patient-days increased by 10% in the surgical department and decreased by 5% in the medical department during the same periods. The decrease from 2009 to 2010 of PPI utilization in CDF comparing to NE is statistically significant: p<0.0001. Use of other antacids didn’t change, ulcers or digestive hemorrhages decreased slightly from 2009 to 2010 in both hospitals.

**Conclusions:** The study showed that with a very low-cost intervention, it is possible to decrease considerably the use of PPIs in a hospital, without taking any risk for gastro-intestinal complications.