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Karel Urbánek
Dear participants,

63. Czech and Slovak Pharmacological Days are taking place again, after six years, in Olomouc, Czech Republic. It is a honor for all enthusiasts who work in the field of pharmacology and related sciences in this city and a pleasure to invite colleagues from both our sister countries, and enjoy the hospitality and friendship of Olomouc and Haná region.

Pharmacology has evolved during last years into a science with many new applications, and, simultaneously, with new challenges and tasks to be solved in the future. Pharmacology and pharmacogenetics is taken as an example of personalization of medicine in the 21st century. Development of experimental techniques has opened possibilities to answer questions about which we only dreamed of in the past. Also, the borders between life sciences seem to be more fuzzy than before which hopefully will contribute to application of approaches formerly typical for each discipline.

The organizers also hope that this Conference will be a good place for exchanging experience in science as well as in the education of pharmacology in general.

Thank you for coming to Olomouc.

Pavel Anzenbacher
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0-1
Ethanol and its principle metabolite acetaldehyde affect inward rectifier potassium current I_{K1} in rat ventricular myocytes

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Alcohol intoxication may induce arrhythmias, most frequently the atrial fibrillation (AF). Increase of inward rectifier potassium currents including the voltage-gated current I_{K1} is known to play an important role in the pathogenesis of AF. Data describing effects of ethanol and its principle metabolite acetaldehyde on mammalian I_{K1} are rare and controversial. Hence, we aimed to analyse I_{K1}-changes in the presence of ethanol and acetaldehyde in enzymatically isolated rat right ventricular myocytes by the whole cell patch clamp technique at room temperature. Ethanol (0.2–200 mM) and acetaldehyde (3–300 μM) were applied by the rapid perfusion system (each concentration in 3–19 cells). A dual effect of ethanol on I_{K1} was observed. At very low concentrations up to 0.8 mM (~0.04‰), ethanol inhibited I_{K1}, however, at concentrations above 20 mM (~0.92‰), ethanol conversely stimulated I_{K1}. The effect was voltage-independent. In accordance with these results, I_{K1}-stimulation was preceded by a transient I_{K1}-inhibition at the beginning of ethanol application. 2 and 8 mM ethanol (~0.09 and 0.37‰ respectively) caused inhibition of I_{K1} in some cells but its stimulation in others. Acetaldehyde inhibited I_{K1} with the concentration causing 50%-inhibition IC_{50} = 61.1±5.1 μM (the Hill coefficient n_H = 1.51±0.18), i.e. I_{K1}-inhibition seems to be negligible in the clinically relevant plasma concentrations of acetaldehyde (in healthy humans usually up to 3.7 μM). We conclude that ethanol exerts a dual effect on the cardiac I_{K1}. Inhibition of I_{K1} in some cells and its stimulation in others might result in the heterogeneity of cardiac repolarization with possible arrhythmogenic consequences. It is unlikely that, in healthy humans, acetaldehyde significantly contributes to the arrhythmogenesis observed after the alcohol consumption.

ACKNOWLEDGMENT

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0-2
Effect of methycobalamin application in patients with autism

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Oxidative stress has been suggested to be one of the key elements in the pathophysiology of autism. An intervention targeted to the glutathione metabolic precursors could improve plasma biomarkers of impaired methylation capacity and improve behavior in patients with autistic disorder. The aim of our project was to examine whether methycobalamin application would affect the glutathione redox status and autistic disorder symptoms. 37 patients with autistic disorder were enrolled. Exclusion criteria for subject selection were: Asperger syndrome, high-functioning autism, epilepsy, selected pharmacotherapy affecting CNS. Oral form of methycobalamin was given daily at dose 500 μg. Venous blood was collected at d0 and d100 and redox status of glutathione and levels of homocystein, cystine and cobalamin were determined. The psychological profile was defined at d0 and d100 by psychologist using scale. Oral application of methycobalamin at the dose 500 μg per day influenced glutathione redox status. Social interaction was increased, including social responsiveness and eye contact. Oral application of methycobalamin in patients with autism seems to potentiate antioxidative mechanisms and leads to the changes in the psychological profile of the patients.

0-3
Molecular forms of cholinesterases in heart

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Cholinesterases are important enzymes that are targeted by various xenobiotics. In pharmacology, cholinesterase inhibitors are used in the diagnosis and treatment of skeletal muscle weakness and the therapy of memory
decline. Recently, importance of cholinesterases in heart has been highlighted by multiple research groups. Moreover, a link between changed enzyme activities and some cardiovascular diseases has been suggested.

Aim of the present project was to characterize and localize molecular forms of cholinesterases in heart.

Genetically modified mice lacking different molecular forms of cholinesterases were used in the project. Cholinesterase activities were determined in heart compartments by Ellman’s method. Different molecular forms were distinguished in biochemical method of sucrose gradient. Precise localization of different molecular forms of cholinesterases in heart was determined in light microscopy by modified Karnovsky and Roots staining. Specific monoclonal and polyclonal antibodies were used to visualize cholinesterases by fluorescence microscopy.

We confirmed the presence of multiple molecular forms of acetyl- and butyrylcholinesterase in mouse heart. The highest acetylcholinesterase activity was determined in atria, with dense localization in sino-atrial nodal region. Molar form ratio was balanced. Butyrylcholinesterase is dispersed in all heart regions, while activity is 2.5-fold higher in ventricles than in atria. Predominant molecular forms of butyrylcholinesterase are monomers/dimers in all studied heart regions.

ACKNOWLEDGEMENT

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0-4

Developmental manipulation of monoaminergic systems affects neurobehavioral and neuroendocrine regulations

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Monoamines have been shown to affect a variety of behavioral functions, such as aggression, sexuality, anxiety, mood or learning. In brain development, they play an important organizational role, including cell division, migration, synaptogenesis, maturation of the cortex and development of neuroendocrine systems. In males, for example, serotonin is markedly reduced during the 2nd and 3rd postnatal week what is essential for full masculinization and defeminization of the brain and behavior. Manipulation of monoaminergic systems by the SSRI and/or SNRI antidepressant drugs represents a risk factor for healthy cognitive, emotional and behavioral development. Drugs of the SSRI/SNRI class belong to the most frequently prescribed antidepressants in the treatment of mood disorders during pregnancy and the postpartum period. An abnormal stimulation of serotonin receptors as a result of an increased synaptic availability of serotonin and impairment of the activity of serotonin transporters during the brain development due to administration of these drugs can lead to functional alterations accompanied by postpartum neurobehavioral dysfunctions. However, there are lack of knowledge on possible adverse effects of SSRI/SNRI drugs on the functional brain development and behavior of the offspring. In our experimental study, we focused on adverse reactions of developmental exposure to venlafaxine (VENF) on early postnatal and neurobehavioral development of rat offspring. Our experimental study with venlafaxine showed that it may interfere with brain development by gender-dependant way and affect neurobehavioral adaptations of rat offspring in a new environment.

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0-5

Iron-chelating agents and acute myocardial infarction: in vitro and in vivo study

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Iron (Fe) is an essential element virtually for all living organisms. However, Fe is able to elevate the production of reactive oxygen species via Fenton chemistry. Moreover, its homeostasis is disrupted in acute myocardial infarction (AMI), which results in the promotion of oxidative stress. Because of the mentioned role of Fe in AMI, which has been the main cause of morbidity and mortality worldwide, the use of Fe-chelating agents for therapy of AMI could have a therapeutic potential.

In this study, Fe-chelating properties, effects on inhibition of Fenton chemistry of flavonoids and synthetic 1-phenyl-3-methyl-4-acylpyrazol-5-ones were evaluated. Moreover, effects of dextrazoxane were analysed in the isoprenaline model of AMI in Wistar: Han rats.

In flavonoids, the most effective substitution was 6,7-dihydroxy group presented in baicalein, which was similarly effective as a standard iron chelator deferoxamine, but its effect on Fenton chemistry was lower. The 3-hydroxy-4-keto conformation together with 2,3-double bond and the catecholic B ring (e.g. in quercetin) were associated with a substantial chelation but its effect on Fenton chemistry was rather negative. In acylpyrazolones, of particular interest is 2,6-bis[4(1-phenyl-3-methylpyrazol-5-one)]carbonyl]pyridine whose Fe-chelating properties...
increased when pH was decreasing. Interestingly, Fe-chelating properties of dexrazoxane did not play the major role in the isoprenaline model. Its protective effects were probably mediated by inhibition of late myocardial impairment and ventricular fibrillation likely due to inhibition of myocardial calcium overload.

Conclusively, detailed in vitro studies are necessary for the assessment of potential use of Fe chelators in the catecholamine model of AMI.
used in monotherapy. Our results demonstrated the significant antiinflammatory effect of Provinol.

In conclusion, we can summarize the most important findings of our experiments: The polyphenolic compound Provinol possesses efficient antiasthmatic activity. Provinol had bronchodilatory, antitussive effect, suppressed asthmatic inflammation of the airways. Furthermore, Provinol amplified the bronchodilatory and antitussive effect of budesonide and theophylline.

ACKNOWLEDGEMENT

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0-8

ACTH mediated regulation of the human MC2R receptor

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ACTH action on the adrenal gland is mediated by its receptor termed MC2R belonging to the superfamily of G-protein-coupled receptors (GPCRs) and to the subfamily of melanocortin receptors (MCRs). MC2R is coupled to heterotrimeric Gs protein leading to cAMP production and activation of the PKA pathway. Upon prolonged agonist stimulation, these receptors classically uncouple from their effector enzymes – a process called desensitization – and undergo internalization using complex cellular machinery.

The aim of this study was to investigate MC2R regulation pattern as well as molecular mechanism(s) involved in these phenomena. C-myc tagged hMC2R was expressed in the M3 (mouse melanoma) cell line enabling to evidence hMC2R both by immunoblotting and by indirect immunofluorescence. Stimulation with ACTH induced production of cAMP with EC(50) values ranging from 7.6-11.9 nM in transient and stable transfectants, respectively. Pretreatment with ACTH induced a dose-dependent loss of cAMP production, maximal desensitization occurred after 15 min of 10 nM ACTH stimulation and was PKA-dependent. ACTH-induced loss of cAMP production was accompanied by receptor sequestration into intracellular vesicles reaching its maximum after 30 min 10 nM ACTH exposure. Immunofluorescence colocalization studies revealed that hMC2R were redistributed in intracellular vesicles through a clathrin-dependent, but caveolae-independent, process involving PKA. In conclusion, the present results indicate that hMC2R undergoes physiologic agonist desensitization and internalization, both processes being PKA-dependent.

0-9

Flavonoids from Morus alba affect cell cycle of human cancer cells and inflammatory response in macrophage-like cells

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The root bark of Morus alba L. (MA) is used for its diuretic, antitussic, antiadibetic, and antipyretic effects in world traditional medicine. Therefore, Morus species plants have been intensively studied from phytochemical point of view and bioactive compounds of flavonoid character have been isolated. The aim of this study was to evaluate cytotoxicity of three flavonoids isolated from MA (kuwanon E, cudraflavone B, and 4′-O-methylkuwanon E), and to determine their effects on proliferation of THP-1 cells, and on cell cycle progression of cancer cells. Anti-inflammatory effects were also determined for all three given flavonoids. From the three compounds tested, cudraflavone B showed the strongest effects on cell cycle progression and viability of tumor and/or immortalized cells, and also on inflammatory response of macrophage-like cells. Kuwanon E and 4′-O-methylkuwanon E exerted more sophisticated rather than direct toxic effect on used cell types. Our data indicate that mechanisms different from stress-related or apoptotic signaling pathways are involved in the action of these compounds. Although further studies are required to precisely define the mechanisms of MA flavonoid actions, here we clearly demonstrate their effects combing antiproliferative and anti-inflammatory activity in human cancer and macrophage-like cells, respectively. Confirmed dual activity of tested prenylated flavonoids could be an inspiration for chemical modifications of their structures or isolation of similar substances in order to get more potent agents usable for clinical practice in future.
ACKNOWLEDGEMENTS

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0-10

**TNFRSF1A and TNFRSF1B gene polymorphisms and their impact on effectiveness of therapy with infliximab**

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The activity of TNFα is mediated by interaction with its receptors (TNFR). Polymorphisms in genes of such receptors are expected to interfere with their functions due to changes in protein structure.

The aim of this study was to evaluate whether polymorphisms in TNFRSF1A and TNFRSF1B genes influence the efficacy of infliximab therapy. A total of 116 Caucasian CD patients treated with infliximab were genotyped. Therapy effectiveness was determined and patients were separated into responders (n=98) and non-responders (n=18). The genotypes of TNFRSF1A (T4672G, G3794C) and TNFRSF1B (T11695C, T587G) was determined by PCR-RFLP.

The frequency of variant alleles of TNFRSF1A was comparable between responders and non-responders. Variant allele TNFRSF1B 11695C was more common in non-responders (41.7% vs. 30.1%). Similarly the frequency of TNFRSF1B 587G allele in non-responders was 33.3% vs. 18.9% on responders. Homozygotes for variant alleles of TNFRSF1B 11695C were found more often (n=5, 27.8%) than in responders (n=6, 6.1%). We suggest that TNFRSF1B 11695C variant allele is associated with low therapeutic effect of infliximab.

ACKNOWLEDGMENT

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**O-11**

**Tolerability of gentamicin in septic preterm neonates with a patent ductus arteriosus**

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**Background.** A patent ductus arteriosus (PDA) is defined as a shunt between the arterial and the venous system which leads to a negative effect on prerenal circulation. This condition has possible consequences to renal and hepatic blood flow. In septic neonates treated with gentamicin (Ge) a PDA may lead to both an impairment of gentamicin clearance and increased vulnerability of the target organs resulting in enhanced Ge toxicity.

**Aims.** The main goal was to assess tolerability of kinetically guided therapy with Ge in critical ill preterm neonates (TDM) and to identify the impact of covariates (hypotension and body fluid retention) on the Ge pharmacokinetics (PK).

**Methods.** This open-label, prospective study (January 2006 - July 2009) enrolled preterm neonates (GA≥34 weeks) during the first week of life stratified in Group 1 (GA >31 weeks) and Group 2 (GA within 34-38 weeks). The first and second doses of gentamicin 4 mg/kg were adjusted according to birth weight at a 48-hour postdose interval, next dosing was kinetically guided as described previously (1). Tolerability as acute renal dysfunction during antibiotic therapy was assessed using laboratory examinations (Sₐ, Sₐmax, EF-Na, EF-Mg, and U-Ca/Ucr). Chronic renal dysfunction monitored over postnatal year 1-5 considered nephrocalcinosis and ototoxicity. Signs of nephrocalcinosis were regularly examined by renal sonography (Acuson Aspen Electric Medical Service). Hearing abnormalities were monitored using transient otoacoustic emission recordings (Danax AS 72 Audiometer Echo-screen TDA, CZ).

**Results.** 54 preterm neonates were enrolled to this study as follows: Group 1 (N=32), 18/32 of them with PDA, Group 2 (N=20), 4/20 with PDA. Neonates with PDA were treated with ibuprofen (15 kg/mg in 3 consecutive days). Mean (SD) gentamicin clearance (CLGe₁) estimated after the first Ge dose = 0.47 (0.09) mL/min.kg⁻¹, in Group 1, and 0.66 (0.10) mL/min.kg⁻¹ in Group 2, was significantly lower in the former (P=0.005). This difference resulted from PDA and treatment with ibuprofen as shown by comparison of CLGe₁ in PDA neonates: 0.370 (0.250) mg/kg.min⁻¹, vs those in non-PDA neonates: 0.700 (0.240) min.kg⁻¹ (P<0.001). These results were
Inhibition of phosphodiesterases (PDE) leads to both of treated with bronchodilating and anti-inflammatory drugs. Chronic inflammatory diseases, associated with airway obstruction and cough, are usually associated with suppression of haematological markers of inflammation and apoptosis in animals treated with roflumilast, suggesting potential use in diseases associated with allergic inflammation.

Conclusions. Kinetically guided dosage of gentamycin based on plasma concentrations after the first dose should take into account comorbidities leading to changes in PK parameters such as a patent ductus arteriosus.

0-12
Influence of roflumilast on in vivo and in vitro airway reactivity and apoptosis in ovalbumin-sensitized guinea pigs
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Introduction. Chronic inflammatory diseases, associated with airway obstruction and cough, are usually treated with bronchodilating and anti-inflammatory drugs. Inhibition of phosphodiesterases (PDE) leads to both of these effects and as the persistence of airway inflammation depends on a decrease in apoptosis of T lymphocytes and eosinophils, PDE inhibitors influence apoptosis of immune cells too. In chronic obstructive pulmonary disease, roflumilast, selective PDE4 inhibitor, has been recently approved for the pharmacotherapy. The aim of this study was to evaluate the effect of long-term administration of roflumilast (p.o. and inh. way of application) in experimentally induced allergic inflammation (model of allergic asthma) in guinea pigs.

Material and Methods. 32 male adult guinea pigs, divided into 4 groups, have been used in the study. Control group has been left without sensitization. The latter 3 groups have been sensitized with ovalbumin over two weeks and thereafter treated perorally for 7 days with roflumilast at the daily dose of 0.5 mg/kg b.w. or by 3 minutes inhalation (5 mg/10 mL), or with vehiculum, respectively. Specific airway resistance measured in whole-body double-chamber plethysmograph has been used as a marker of in vivo airway reactivity. The in vitro reactivity of tracheal and lung smooth muscle has been tested using organ bath method.

Results and Conclusion. Sensitization with ovalbumin has led to significant increase in in vivo and in vitro airway reactivity. Roflumilast reduced both specific airway resistance after nebulisation of histamine, and in vitro airway reactivity to cumulative doses of acetylcholine in tracheal and lung tissue strips. These changes have been associated with suppression of haematological markers of inflammation and apoptosis in animals treated with roflumilast, suggesting potential use in diseases associated with allergic inflammation.

0-13
Preclinical studies of a novel derivative of quercetin–inhibitor of aldo-ketoreductases AKR1B1 and AKR1B10. Implications for chronic diabetic complications, inflammatory disorders and cancer
Ivana Milacková, Marta Soltesova-Prnova, Magdalena Majeková, Ruzena Sotnikova, Jana Navarova, Lucia Rackova, Beatriz Diez-Dacal, Dolores Perez-Sala, Shabnam Enayat, Sreepama Banerjee, Milan Stefek

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Acknowledgement
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properties not violating Lipinski’s rules, indicate good bioavailability of CHNQ with prospective pharmacological application.

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0-14

The role of nicotinic receptors in heart physiology

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Cardiac function is controlled by autonomic nervous system. The autonomic imbalance with reduced vagal and increased sympathetic activity is associated with cardiac diseases and increased risk for cardiac mortality. Experimental reports have demonstrated that stimulation of vagal nerve is able to improve outcomes for diseases like hypertension or heart failure. Vagal activity of heart is probably disrupted in ganglion for these diseases, where the transmission is mediated by nicotinic receptors (nAChR). Neuronal nAChR can be formed by various combinations of heterologous subunits that determine ionic and ligand binding characteristics which are important for physiological functions. The aim of this project was to study the role of α7, β2 and β4 nAChR subunits in heart physiology.

In our in vivo and in vitro experiments, mice with absence of α7 (α7−/−), β2 (β2−/−) or β4 (β4−/−) subunits and wild-type mice were used. In vivo hemodynamic measurements were performed at physiological conditions and during β1-adrenergic stimulation by dobutamine. In vitro experiments were performed on isolated heart perfused by Langendorff in physiological conditions and during β1-adrenergic stimulation by neostigmine. Cholinergic stimulation was antagonized by atropine or hexamethonium.

In heart of α7−/− mice, the effect of neostigmine was impossible to antagonize by atropine or hexamethonium, suggesting change of muscarinic receptor signalling. As no changes in heart physiology were observed in β2−/− mice in vivo or vitro, we assume that β2 subunits do not have an obvious function in heart physiology or that an effective adaptation to the lack of this subunit occurred.

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0-15

Elucidation of the transformation of nabumetone to the active metabolite, 6-methoxy-2-naphthylacetic acid (6-MNA)

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Nonsteroidal anti-inflammatory prodrug nabumetone is after oral administration converted in liver to 6-methoxy-2-naphthylacetic acid (6-MNA), the principal metabolite responsible for the NSAID effect. No intermediates between nabumetone and 6-MNA have been identified so far. In our recent study (Nobilis M. et al., J Pharm Biomed Anal 80, 164-172, 2013), a new, as yet unreported phase I metabolite was identified within a thorough study of nabumetone metabolism by human and rat liver microsomes. Extracts from these biomatrices were subjected to chiral HPLC-PDA and achiral HPLC-MS/MS analyses to elucidate the chemical structure of this metabolite. The new metabolite (having the elemental composition C_{14}H_{16}O_{3}) was identified as 4-(6-methoxy-2-naphthyl)-3-hydroxy-butan-2-one (3-hydroxy nabumetone) and its identity was confirmed by the synthesis of the compound. Following the incubation of nabumetone with the rat and human liver microsomal fraction, 3-hydroxy nabumetone was formed, but no 6-MNA was detected in this biomatrix. On the other hand, when 3-hydroxy nabumetone was incubated with isolated rat hepatocytes, 6-MNA was detected as the principal metabolite. Hence, 3-hydroxy nabumetone was found to be the missing link in nabumetone biotransformation to 6-MNA.
On the molecular pharmacology of oxidative burst inhibition in human neutrophils

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Neutrophils are present in high numbers in areas of inflammation, where they constitute an important source of reactive oxygen species (ROS). The massive production of antimicrobial and tumoricidal ROS in an inflammatory environment is called “oxidative burst” and plays an important role as the first line of defense against environmental pathogens. There are at least two signalling pathways responsible for induction of neutrophil activation: one is the protein kinase C (PKC)-mediated pathway stimulated with phorbol-4ß-12ß-myristate-13α-acetate (PMA), and the other is the Src family protein tyrosine kinase-mediated pathway. Resveratrol (RES), a polyphenolic phytoalexin, is one of the most extensively studied natural products, with wide-ranging biological activity and tremendous clinical potential. RES has been shown to have antioxidant, anti-inflammatory, anti-proliferative, and anti-angiogenic effects, while those on oxidative stress possibly being presumably the most important.

Oxidative burst of whole human blood measured by luminol/isoluminol-enhanced chemiluminescence (CL) and stimulated with PMA, fMLP (N-formyl-methionyl-leucyl-phenyl-alanine), OpZ (opsonized zymosan) and Ca2+-ionophore A23187 was inhibited in a concentration-dependent way, indicating suppression of both receptor and nonreceptor activated CL by resveratrol. Results from isolated human neutrophils revealed that resveratrol was active extracellularly as well as intracellularly in inhibiting the generation of reactive oxygen species. Liberation of ATP and analysis of apoptosis showed that in the concentrations of 100 μM significantly decreased PMA-induced phosphorylation of PKC α/βII.

The results suggest that resveratrol represents an effective naturally occurring substance with potent pharmacological effect on oxidative burst of human neutrophils. It should be further investigated for its pharmacological activity against oxidative stress in ischaemia-reperfusion, inflammation and other pathological conditions, particularly neoplasia.

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Kinetically guided dosage of gentamicin in neonates treated for perinatal asphyxia

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Background. Modest therapeutic whole body hypothermia (HT) is frequently used in neonates with moderate and severe hypoxic-ischemic encephalopathy (HIE). If the neonate was contemporary treated with gentamicin (Ge) for sepsis, this new condition might modify the Ge pharmacokinetics (PK) and consequently its bactericidal efficacy.

Aims. to analyze the PK of Ge in septic neonates suffering from perinatal asphyxia (PA) and treated with HT during the first week of life.

Methods. This open-label, prospective study (January 2006 - December 2009) enrolled full-term and nearly term neonates admitted to the Neonatal Intensive Care Unit (NICU). The neonates were treated with Ge for suspected or proven sepsis and those with PA underwent HT (the whole body temperature within 33-34 °C). The first and second doses of gentamicin (4 mg/kg) were adjusted according to birth weight at a 48 - hour postdose interval, next dosing was kinetically guided as described previously (1).

Results. 54 full-term/nearly term neonates (birth weight 2.5-4.56 kg; GA within 36-42 weeks) were divided to 4 groups: 3 groups of neonates suffering from sepsis and PA were treated as follows: group 1 (14/54) with Ge

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and HT (within 24 hours after birth); group 2 (8/54) with Ge and HT (within 24-72 hours after birth); group 3 (11/54) with Ge only. Septic neonates without PA (group 4) were given Ge (21/54). PK parameters of Ge based on 4 plasma concentrations after the first dose were as follows: mean (SD) of Ge clearance of group 1-4 reached 0.57 (0.24) mL/min.kg⁻¹, 0.81 (0.48) mL/min.kg⁻¹, 0.63 (0.27) mL/min.kg⁻¹, and 0.91 (0.41) mL/min.kg⁻¹, respectively. This value was significantly lower in group 1 vs group 4 (P=0.004). Mean (SD) values of Vd Ge in group 1-4 was 0.32 (0.15) L/kg, 0.41 (0.18) L/kg, 0.36 (0.19) L/kg, and 0.55 (0.40) L/kg, respectively. This PK parameter was significantly higher in group 4 vs group 1 (P=0.045).

Conclusions. Dosage of gentamicin should be individualized according to maturational/pathophysiological covariates which might influence the gentamicin PK.

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Diagnostics of resistant forms of tuberculosis – conventional vs. molecular-genetic methods

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Despite tuberculosis (TB) currently belongs to rarer respiratory diseases in the territory of Slovak Republic ranking among countries with its low incidence, the incidence in some other countries is growing year by year. More serious is the fact that there appear mycobacterial strains resistant to various anti-tuberculosis drugs (AT) more frequently. The emergence and spread of multi-drug resistant tuberculosis (MDR - TB) and extensively drug-resistant tuberculosis (XDR - TB) are major challenges for global tuberculosis control, since the treatment of resistant forms is difficult both medically and financially.

In Slovakia, the diagnosis has been relying on standard procedures based on the cultivation of mycobacteria and subsequent drug susceptibility testing to antituberculosis (AT) for many years. Since these methods require a lot of time, many times exceeding the time of the initial phase of tuberculosis treatment onset, it is necessary to use new, time-saving diagnostic options. One of the possibilities is an automatic system that speeds up the time of cultivation, and the time needed to determine sensitivity to AT. Even faster and more modern way is the use of molecular-genetic methods, allowing direct evidence of M. tuberculosis in a biological, or culture material, respectively. Furthermore, with these methods, the evidence of resistance to the two most important anti-tuberculosis drugs - Isoniazid and Rifampicin with MDR - TB, and ethambutol, aminoglycosides and fluoroquinolones in XDR – TB is enabled.

Conventional cultivation methods with drug susceptibility testing to AT are slow and complicated, requiring several consecutive steps for diagnosis. During this time patients may be treated inappropriately, drug resistant strains may continue to spread, and amplification of resistance may occur. Therefore rapid diagnosis and identification of MDR-TB or XDR-TB strains are prerequisites for the worldwide fight against TB.

0-19

Issues of oral anticoagulants prescription in elderly patients with atrial fibrillation

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Atrial fibrillation (AF) represents a condition that is associated with 5-fold increase of the risk of stroke compared to healthy population. The risk of stroke increases with advancing age, as well. Consequently, elderly people with AF are an eligible group for prophylactic anticoagulant therapy. Benefit of oral anticoagulants in patients with AF has been confirmed in all age groups. However, several observational studies showed a substantial underutilization of oral anticoagulants in elderly populations with AF. CHADS₂ and CHA2DS₂-VASc score systems were created for evaluation of the risk of stroke in individual patient with AF.

We carried out an observational study to determine the influence of the risk factors for stroke defined by CHADS₂ and CHA2DS₂-VASc score on the underutilization of oral anticoagulant treatment. In our study, 111 patients aged ≥ 65 years with AF without any contraindication for such therapy were included. There was no difference in the values of CHADS₂ and CHA2DS₂-VASc score in the group of warfarin users and non-users. We found significant difference in prescription of anticoagulant treatment between elderly men and women with AF (P<0.001).
Aryl hydrocarbon receptor and its crosstalk with glucocorticoid receptor in human placental barrier

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Glucocorticoids (dexamethasone and betamethasone) are transplacentally administered before 35 weeks of pregnancy in fetuses with the risk to be born prematurely to increase fetal pulmonary surfactant production and prevent infant respiratory distress syndrome.

Aryl hydrocarbon receptor (AHR) is a mediator of the toxicity of particular xenobiotics such as dioxin, polycyclic aromatic hydrocarbons and halogenated biphenyls that are involved in tumor initiation and progression. The CYP1A1 is the most important xenobiotic-metabolizing cytochrome P450 enzyme in the human placenta. Importantly, CYP1A1 generates reactive species and its placental activity is elevated in smoking women. CYP1A1 expression is mainly controlled by aryl hydrocarbon receptor ligands.

We studied the effects of the glucocorticoids on inducibility of AHR, ARNT, CYP1A1 and other AHR target genes (CYP1A2, CYP1B1, UGT1A1, BCRP) by prototype AHR ligand 3-methylcholanthrene (3MC) in isolated human placental trophoblast culture.

We showed that glucocorticoids alone had no effect on activity and protein/mRNA expression of CYP1A1 and little effect on mRNA expression of other AHR target genes. However, glucocorticoids significantly augmented EROD enzymatic activity and significantly stimulated only CYP1A1 mRNA induction mediated by 3MC.

In conclusion, our data suggest that glucocorticoids have no effect on AHR target genes expression per se, but they may potentiate CYP1A1 induction in human term placental trophoblast.

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Application of computer-based modeling in the analysis of cardiovascular regulation mechanisms

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Introduction. Even after the replacement of analog recorders of vascular segment responses to vasoactive stimuli by digital ones evaluation of vessel reactivity still relies mainly on several classical descriptive parameters such as amplitude of contractile responses and area under the curve. Advanced current digital measurement circuits allow incomparably more accurate assessment of a wide set of contractile response parameters, whose determination was not possible by routine descriptive methods.

Aim. To quantify and assess computer-based model parameters of digitally recorded contractile responses of perfused rat arterial segments in comparison with parameters available by conventional descriptive evaluation.

Material and Methods. Our first study analyzed responses of rat renal arterial segments from control and diabetic animals to successively increasing bolus doses of noradrenaline (0.1; 0.5; 1; 3; 6; 10 μg). Finally, after precontraction there was induced a relaxation by single bolus dose of acetylcholine (20 μg). Our second study compared segment responses of renal arteries from healthy rats induced by noradrenaline and adrenaline under the same pattern of stimulation. For the analyses descriptive evaluation were used as well as computational modeling using methods Levy and Monte Carlo.

Results. At descriptive evaluation of contractions we found no significant difference between groups at any used dose of neither noradrenaline nor adrenaline. Relaxatory responses in the diabetic group showed distinct decline compared to controls. Digital modeling procedures revealed significant changes of several characteristic parameters of contractile responses in the diabetic group vs. controls and also in responses to noradrenaline vs. adrenaline.

Conclusion. The used software design allows to quantify large scale of contractile response parameters unavailable for descriptive evaluations. Thus it seems to be an useful tool in comparison with routine descriptive methods.

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The specific issues in pharmacotherapy in elderly patients – misprescription and underprescription

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The specific features of pharmacotherapy in elderly people results from changes in pharmacokinetics and pharmacodynamics of drugs, polymorbidity, polypharmacy as well as altered compliance. The use of certain drugs is accompanied with increased risk of adverse drug reactions when administered in elderly patients. For this reason lists of potentially inappropriate medications were created. For drugs listed in these lists safer or more effective alternatives are available. The issue of excessive, often potentially inappropriate and also duplicate drug prescription is widely discussed in the literature. On the other hand, less attention is paid to the issue of the absence of beneficial medication administration. This is a more frequently occurring issue in comparison with potentially inappropriate drug prescription. The consequences of the absence of administration of beneficial drugs may manifest later than the adverse drug reactions of potentially inappropriate medications. The consequences of absenting prescription are very serious, e.g. the increased incidence of cardio- and cerebrovascular events. A certain fear of doctors regarding possible adverse drug reactions represents an important reason for non-administration of beneficial medicines.

Cardiovascular protective treatment represents an example of relatively often underused therapy. In our study, 167 patients aged ≥65 years with acute myocardial infarction or stroke and patients with history of these events were included. Antiplatelet medication was absent in 52 (31.1%) patients without contraindication for antiplatelet therapy. Underprescription of antiplatelet therapy was common in patients with stroke ($P<0.001$).
Association of haplotypes HLA-DQ2, HLA-DQ8 and polymorphism G-308A IN TNF-alpha gene with coeliac disease

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Coeliac disease (CD) is one of the most common autoimmune diseases but it is estimated that only 10% of patients are diagnosed. Typical abdominal symptoms most commonly prevail in children; in adults, usually exhibit atypical symptoms (anemia, osteoporosis, dermatitis herpetiformis etc.), which are harder to diagnose.

Genetic predispositions play an important role in the pathogenesis of CD. CD is associated with HLA-DQ2 haplotype (coded DQA1*05/DQB1*02) and HLA-DQ8 (DQA1*03/DQB1*0302). Other genes that could be involved in the development of CD are studied, of which the TNF-α gene is a candidate gene.

The aim of this study was to create, validate and standardize PCR-multiplex methodology for the detection of CD risk haplotypes HLA-DQ2 and HLA-DQ8 and determination of G-308A polymorphism in TNF-α gene by PCR-RFLP. Genotypization was performed in 126 patients with confirmed diagnosis of CD, 66 family members and 58 healthy subjects. The association between the aforementioned genetic variants and the occurrence of CD was also explored.

Statistical analysis confirmed significant differences in the occurrence of:
- HLA-DQ2 haplotype among patients and healthy subjects (P=0.0001)
- HLA-DQ2 haplotype among patients and family members (P=0.0308)
- HLA-DQ2 haplotype among family members and healthy subjects (P=0.0001)
- HLA-DQ8 haplotype among HLA-DQ2 negative and HLA-DQ2 positive patients (P=0.0156)
- variant allele 308A among patients and healthy subjects (P=0.0001)
- variant allele 308A among patients and family members (P=0.0188)
- variant allele 308A among family members and healthy subjects (P=0.0046)

Our results confirmed the association of haplotypes HLA-DQ2, HLA-DQ8 and G-308A polymorphism in the TNF-α gene with coeliac disease.

BKCa channels and experimental allergic asthma

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BKCa are universally expressed in organism and they participate in various physiological processes. The channels are activated by both depolarization as well as by calcium concentration increased. BKCa channels play an important role in airway smooth muscle (ASM). They can be an important regulator of membrane potential and integral signal in ASM. Regard to above mentioned, this channel activation becomes possible mechanism for the asthma treatment.

The aim of presented studies was to prove whether long-term therapy by opener of BKCa ion channels NS1619 inhibit the cough reflex, modulate the ASM reactivity and influence levels of exhaled NO and mast cells infiltration in conditions of experimental allergic inflammation of the airways in guinea pigs.

Presented studies were carried out after treatment of ovalbumine-sensitized guinea pigs by NS1619 for 14 days. Allergic inflammation of airways was induced by repetitive exposure of guinea pigs to ovalbumine and the degree of allergic inflammation was determined by histological analysis of tracheal and pulmonary samples. The cough reflex was induced by 0.3 M citric acid aerosol for 3 min interval, in which total number of coughs was counted. ASM reactivity in vivo was expressed as values of specific airway resistance (sRaw) calculated by Pennock. The influence of NS1619 on exhaled NO levels and degree of mast cell infiltration visualized by tryptase positivity was used to prove its anti-inflammatory activity.

NS1619 significantly reduced sRaw values after long-term application regardless to used bronchoprovoking substances but this opener didn`t influence cough reflex. Immunohistochemical staining of specimens showed increasing signs of allergic inflammation during sensitization procedure as well as significant anti-inflammatory effect of NS1619. These results were confirmed by indicative measurement of exhaled NO levels.

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Improvement of standard anti-arthritic therapy with N-feruloylserotonine addition to methotrexate in rat adjuvant arthritis

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Most anti-rheumatics have side-effects when used in higher doses and/or within long-term dosage. Methotrexate (MTX) has become the main immuno-suppressive substance used in the treatment of patients with rheumatoid arthritis (RA). N-feruloylserotonine (N-f-5HT) a plant derived natural substance, was found to possess many positive biological effects: antiproliferative, antioxidative, antiatherogenic as well as inhibition of oxidative burst of isolated neutrophils in whole blood. Combination therapy is expected to have a higher efficacy without toxicity. The aim of this study was to evaluate the effects of N-f-5HT in monotherapy and in combination therapy with methotrexate (MTX) on disease progression in rat-induced adjuvant arthritis (AA).

AA was induced by a single intradermal injection of Mycobacterium butyricum in incomplete Freund’s adjuvant to male Lewis rats. The experiments included healthy animals, arthritic animals without any drug administration, arthritic animals with administration of N-f-5HT in the oral daily dose of 15 mg/kg b.w., arthritic animals with administration of methotrexate in the oral dose of 0.3 mg/kg b.w. twice a week and arthritic animals with administration of the combination of N-f-5HT and MTX. The following parameters were monitored: clinical (change of hind paw volume and arthritic score), biochemical (the activity of cellular γ-glutamyltransferase in spleen and joint homogenates, activity of glutation reductase in brain homogenate and plasmatic levels of TBARS) and immunological (plasmatic levels of CRP, MCP-1, IL-1 and IL-17). Our results showed the beneficial effect of N-f-5HT in combination therapy with MTX. The combination is more effective than MTX alone and it could improve the treatment of RA.

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How to prevent adverse drug interactions in clinical practice

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Polypharmacy in intensive pharmacotherapy presents a typical face of health care in developed countries. It is known, that long term usage of more than 5 medicines probably produce one adverse interaction. In risky groups as elderly patients, others with chronic cardiovascular diseases and DM-2 type patients with complications exceeds consumption more than 10 preparations. Various, mostly pharmacopeidemical studies revealed possibility of 30 to 50% their prevention. These facts forced to build an effective system of predictive drug selection in therapy with the lowest interaction potential. Projection and development of an innovative drug involves a search for identification of possible interactive potential on both levels: experimental and clinical. Preregistration randomized studies of IIb involve evaluation of risk and efficacy of new drug in treatment schedule containing perspective combinations.

The most important data with regard to possible interactions in pharmacotherapy are expected to come from PAAS (Post authorization safety studies), see details in new legislative from GVP, Commission implementing regulation (EU) No 520/2012.

Monitored signals of adverse interactions from these sources will be further validated in special conditions and widely published in EMA, WHO specialized journals.


The most effective prevention of adverse interactions will be achieved by implementation of recent results of pharmacovigilance and its effective communication among health care professionals, physician, pharmacists on one side and patients on the other side.
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Commonly measured biomarkers of oxidative stress do not reliably predict cardiovascular impairment

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Oxidative stress has been linked with cardiovascular diseases in most studies although the pathophysiological relevance has still remained not fully understood. Therefore the aim of this study was to establish the relationship among different commonly used biomarkers of oxidative stress and cardiovascular dysfunction in experimental rats.

The data from 145 male Wistar:Han rats, previously published in original papers analysing effects of iron chelators on a model of acute myocardial infarction (AMI), were re-analysed in this study. A pathological state similar in many aspects to AMI in humans was induced in a half of these animals by administration of isoprenaline (100 mg.kg⁻¹ s.c.). Animals were pre-treated with different iron chelators or solvents in order to mimic treated and non-treated population with and without cardiovascular disorder.

As expected, serum cardiac troponin T (cTnT) correlated strongly negatively with the cardiac function and positively with myocardial calcium levels and wet ventricles weight. Concerning parameters of oxidative stress, only weak negative associations were found for cTnT and total blood glutathione or serum vitamin C concentrations, while no significant correlations were found with serum vitamin E and plasma TBARS. Similar findings were documented between other parameters of cardiac function and biomarkers of oxidative stress.

Conclusively, it appears that commonly used biomarkers of oxidative stress may not reliably correspond to the cardiovascular dysfunction.

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0-29
Polymorphisms in protein C gene promoter region and endothelial protein C receptor gene as predisposing factors for venous thrombosis

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The primary abnormalities that are associated with an increased risk of venous thrombosis (VT) are the inherited deficiencies of protein C. Protein C (PROC), encoded by the PROC gene, acts through its high affinity for binding to its transmembrane receptor (EPCR) encoded by the EPCR gene. The objective of the present study was to analyze the link between haplotypes of three polymorphisms in the promoter region of PROC gene, the polymorphism in the EPCR gene and the occurrence of VT.

We genotyped 289 individuals - 126 cases with documented VT and 163 healthy volunteers without a history of DVT. Genetic analysis was carried out in Laboratory of pharmacogenomics, Dept. of Human Pharmacology and Toxikology, Faculty of Pharmacy, UVPS, Brno. The occurrence of three polymorphisms in the promoter of PROC gene (-1654C/T, -1641A/G and -1476A/T) was determined by a single PCR reaction, where -1654C/T and -1476A/T were detected by PCR-REA and -1641A/G by allele-specific probes with Real-time PCR. The 6936A/G polymorphism in the EPCR was detected by PCR-REA.

The groups of patients and controls were simultaneously genotyped for 5 other polymorphisms, which are significantly linked to a predisposition for VT, i.e. the factor V Leiden.

Our results showed that, based on the available knowledge, the combination of TAA haplotypes is associated with a higher plasmatic level of PROC and therefore with a lower risk of the development of VT. This combination occurred with a higher frequency in the control (P=0.0206). Furthermore, we confirmed a significantly higher (P=0.0066) occurrence of the combination of TAA haplotypes and standard genotype AA EPCR in the control than in the group of patients. To the contrary, the frequency of PROC, CAA and CGT haplotypes was insignificantly higher in the group of patients than in the control. Compared with the genetic variant associated with higher PRO levels (TAA), plus the EPCR AA genotype, the genetic variant associated with lower protein C levels (CGT and CAA) plus the EPCR AG genotype is indeed a risk factor for thrombosis.
Current challenges of body weight based intravenous busulfan dosing versus dose adjustment based on therapeutic drug monitoring

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Introduction. Busulfan in high doses is often used to substitute total body irradiation for bone marrow or haemopoietic stem cell transplantation (HSCT) conditioning therapy. Its considerable pharmacokinetic variability and worrying adverse effect in case of extreme exposure warrants pharmacokinetic monitoring in both oral and intravenous forms. Previous works suggested that test pharmacokinetic study enables dose prediction for all of the rest doses given every 6 hourly for four days. However, extensive case series observations and further studies indicate that the drug shows intra-individual variability challenging the first dose based prediction.

Objectives. The principal aim of this communication is to describe typical cases, where dose prediction based on post initial doses concentrations measurement and consequent area under the concentration versus time curve (AUC) calculation may not be reliable and that may endanger the principal goal of the intervention.

Patients and Methods. Three exemplary out layer cases have been processed from patients after the provision of informed consent and approval by an independent ethics committee. An adult male at age of 49 years, and two children (1 male and 1 female) both at age of 2 years, respectively have been treated with i.v. busulfan doses on 2-4 hour lasting infusion (peak), immediately after the end of absorption (trough) followed by samples immediately before the 5\textsuperscript{th} dose given every 6 hourly for four days. However, extensive case series observations and further studies indicate that the drug shows intra-individual variability challenging the first dose based prediction.

Results. At initial measurement AUC in adult patient was and in a female infant case revealed unacceptably low exposure expressed by low AUC 496.4 μg/L-hr and 1284 μg/L-hr, respectively. In contrast, AUC C\textsubscript{trough}C\textsubscript{6} calculated according to the trapezoidal rule in the male child revealed overexposure expressed by AUC C\textsubscript{trough}C\textsubscript{6} 11135 μg.L-hr, which is evidently beyond myeloablative target AUC of 5000-7000 μg/L-hr. All the three patients required another follow-up monitoring with or without dose adjustment.

Conclusions. Our results demonstrate that even after i.v. busulfan administration, individual as well as intra-individual PK/PD variability is of great concern. The overall conclusions drawn from these case series observation is to recommend inter-dose follow-up therapeutic drug monitoring instead of relying on initial dose predictions as highly required tool to guarantee aimed target with careful interpretation of drug levels considering all influential factors. Thus, it is strongly suggested that follow-up AUC monitoring between doses is may certainly help to reduce the risk of poor outcomes both in adult and paediatric HSCT patients.

First clinical pharmacology department in the world

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In the past we have published on the topic of the history of clinical pharmacology, specifically on the life and work of prof. MUDr. Emil Starkenstein who was a professor of pharmacology at Charles University in Prague and who is considered to be one of the founders of the field of Clinical Pharmacology.

Recent serendipitous finding in the Austrian medical journal Therapeutische Monatshefte from 1916 however led us to reconsider the authorship of the term “clinical pharmacology”. It appears that Dr. Hans Januschke, born in Opava in 1883, was tasked by then head of the Pharmacology Department, prof. Hand H. Meyer, to found a dedicated department of Clinical Pharmacology at the Karolinen-Kinderspital in Vienna as early as 1911. The children hospital was then headed by prof. C. Frh. v Pirquet, an interesting figure in his own right.

The department published several case reports on children whose treatment was based on translation of basic pharmacology research. The department also had an experimental unit where Januschke continued his animal research. Literature search did not reveal the fate of the department and none of the Austrian pharmacology departments seem to be claiming to be a successor of what appears to be the first Clinical Pharmacology department in the world. The literature trail ends in 1924.

The authors plan on publishing an account of the history in hope that someone knowledgeable of the ultimate fate of the department will help us write the ending to an important chapter from the very beginnings of our field.
5-Fluorouracil (5-FU) is one of the most commonly used antineoplastic drugs in the therapy of breast, oesophagus, head and neck and especially colon cancer. Hand-foot (HF) syndrome (palmar-plantar erythrodysesthesia) is an adverse effect frequently related with long term i. v. administration of 5-FU and its orally applicable prodrug capcitabine. According to histology findings it can be characterized as non-specific toxic reaction of keratinocytes to the presence of cytotoxic agent and its severity can lead even to the interruption of an effective therapy.

Experimental practice in some clinics showed that topical application of 10% uridine ointment is suitable for curing HF syndrome. This study is to find out if there is evidence for this protective activity of uridine in human keratinocytes cultured in vitro. We also tested cytidine, deoxyctydine, thymidine and their combinations for the ability to protect the cells against 5-FU effect.

We measured cellular viability time progression by recording cellular adherence with RTCA (real-time cell analyzer). We also measured metabolic activity of cells recording cellular adherence with RTCA (real-time cell analyzer). We also measured metabolic activity of cells

The protective effect of pyrimidine nucleosides and their combinations on HaCaT keratinocytes treated with 5-FU: MTT, NTCA and RTCA tests

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P-1

Interaction of rocuronium with human liver cytochromes P450

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Rocuronium is a neuromuscular blocking agent acting as a competitive antagonist of acetylcholine on nicotinic receptors of neuromuscular plate. It is used during balanced anesthesia to induce and maintain the neuromuscular blockade. Its advantage is a relatively fast onset (1 min) and an intermediate duration of its action (30 to 50 min). It is an aminosteroid derivative with quaternary nitrogen atom, with small amount (several per cent of the dose) metabolized to an N-desallyl and 17-desacetyl derivatives. There are indications that this drug may interact with liver microsomal system of cytochromes P450 (CYP) as e.g. an increase of rocuronium effect by macrolide antibiotics; however, no systematic study on this subject has been published in the literature. In this report, results of an in-vitro inhibition of enzyme activities specific for eight individual liver microsomal CYP forms are presented. As the patients are often premedicated orally with diazepam, possible interaction of diazepam with rocuronium has been also studied, namely, the formation of two main diazepam metabolites, has been followed in the absence as well as in the presence of rocuronium.
Results have indicated that rocuronium is able to interact with human liver microsomal cytochromes P450 by binding to the substrate site hence increasing the high spin form of this enzyme. Further studies have shown a concentration dependent inhibition of human liver microsomal CYP3A4 form down to 42% with the highest rocuronium concentration, 189 microM. This effect has been confirmed with two substrates of this enzyme, namely, with testosterone (formation of 6beta-hydroxytestosterone) as well as with diazepam (temazepam formation). Other microsomal CYP forms (CYP1A2, 2A6, 2B6, 2D6, 2E1) have not been inhibited by this drug; however, the CYP2C9 and CYP2C19 activities were inhibited down to 75-80% of their original activity by the highest concentration of rocuronium.

To prove the possibility of rocuronium interaction with other drugs, namely, with diazepam, the effect of rocuronium on formation of two main diazepam metabolites, temazepam (by CYP3A4) and nordiazepam (by action of CYP2C19) in primary culture of human liver hepatocytes has been examined. Rocuronium has caused an inhibition of both these reactions by 15 and 12%, respectively.

In conclusion, the results open a possibility that interactions of rocuronium with drugs metabolized by CYP3A4 (and possibly also CYP2C19) may be observed. On the other hand, the effects described here indicate that the clinical importance is probably limited and that a modulation rather inhibition of the metabolism of these drugs is observed.

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P-2

**Antihypertensive and negatively chronotropic effect of new -ethyl substituted arylcarbonyloxyaminopropanole derivates**

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Department of Chemical Drugs of the Faculty of Pharmacy of Veterinary and Pharmaceutical Sciences in Brno developed new potential ultrashort-acting beta blockers. These compounds 2FC2a, 2FC2b were used in this experiment to pharmacologically evaluate their effect on systolic blood pressure and heart rate in normotensive rats by invasive method. For ultrashort-acting effect is responsible ester bond in the lateral chain of tested compounds. This group is rapidly hydrolyzed by esterases in red blood cells or by free carboxylesterases in plasma (Mokrý P. et al., 38. konf. Syntéza a analýza léčiv, sborník HK, 2009:153).

The experiment was performed in vivo with 21 male Wistar laboratory rats and tested group was divided into 3 subgroups: All subgroups (n=3) were administered the dose of 6.0 mg.kg⁻¹ of 2FC2b, 4FC2b or placebo. The tested agents were administered into vena jugularis and the values of systolic blood pressure and heart rate were monitored for 15 min following the administration. For systolic blood pressure invasive monitoring, the „HSE UNIPER UP-100“ by Hugo Sachs Electronic company, and for the heart rate the „EKG for veterinary purposes“ were used. Tested agents were compared to placebo. 2FC2b caused statistically significant decrease (P<0.01) in systolic blood pressure from 1st up to 2.5th minute and from 3rd to 3.5th minute (P<0.05). Compound 4FC2b only in 0.5th minute (P<0.01). Effect on the heart rate of the substance 2FC2b was without significant response but heart rate was significantly decreased from 0.5th to 3rd minute (P<0.05) by compound 4FC2b.

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P-3

**Drug related problems identified in a community pharmacy during point-of-care testing**

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Point-of-care testing (PoCT) is a part of professional practice in community pharmacies in Slovakia. The aim was to identify drug related problems (DRPs) associated with lipid-lowering treatment in patients who had their biochemical parameters tested in a community pharmacy of the Faculty of Pharmacy in Bratislava, and to classify these DRPs according to the PCNE Classification for DRPs V6.2.

PoCT had been provided to 88 patients (mean age 64.6 years) in period 11/2011 to 11/2012 Most (88.6%) of these patients had been tested repeatedly, which resulted in 169 visits in total. There were 40 DRPs associated with lipid-lowering therapy identified, 24 (60.0%) during the first visits and 16 (40.0%) during the check-up visits. The most common DRP was a suboptimal effect of therapy (code P1.2 according to PCNE V6.2) in 15 (37.5%) cases. In 10 (25.0%) cases there was a potential problem of untreated indication (P1.4). In 9 (22.5%)
cases the therapy was uneffective (P1.1), mostly because of not taking prescribed lipid-lowering drug. Nonallergic adverse drug event (P2.1) of statins such as muscle pain was detected in 5 (12.5%) cases and allergic adverse drug event (P2.2) in one (2.5%) case. Six (15.0%) DRPs did not require an intervention (I0.0). In other cases of DRPs the interventions were either patient consultation (I2.1), referral to physician (I2.3) or both. Three (7.5%) DRPs were solved completely (O1.0) and 4 (10.0%) partially (O2.0). In 5 (12.5%) cases the DRP was not solved due to lack of cooperation of patient (O3.1). The outcomes of the rest cases are not known yet (O0.0) as the project still continues.

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P-4
The real-time radioimmunoassay can enhance the better detection of ligand competitive reactions in vitro

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The competitive assays enable to determine the binding characteristic of new prepared ligands such as radio-labeled monoclonal antibodies as the first step in their pharmacology testing. The better approach lies in in vitro testing, which serves as the better testing system closely modeling the living organism. However, the traditional methods for ligand binding characterization are time and material demanding. Disadvantages coming from the well-established methods can be circumvented with the employment of the real time radioimmunoassay on previously introduced machine LigandTracer.

The real time detection competitive study ran in the arrangement of the pre-incubation of cells with radioiodinated natural ligand for some time and then followed the addition of unlabeled non-natural ligand (either monoclonal antibodies or another natural ligand). The binding domain of epidermal growth factor receptor (EGFR) was used as targeted protein for ligands. The study was performed in vitro with the employment of cancer cell lines expressing EGFR in high density.

The evaluation of measured results revealed the binding ability of employed unlabeled ligands in the competition with the radioiodinated natural ligand. The pre-bound natural ligand was displaced with either low concentrations or high concentrations of the competitors or was not in one case.

On the basis of obtained results, the novel automatic technique has proved its capability to be skillful method for the labeled ligand versus unlabeled ligand competition analysis. Moreover, this technique exceeds well-introduced methods for ligand binding characterization thanks to its measurement simplicity and low cost runs.

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P-5
The biological effects of flavonoids pomiferin and osajin in experiment

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The flavonoids pomiferin and osajin were extracted from infructences of Maclura pomifera, Moraceae.

The aim of the studies was to analyze effects of both substances under the conditions of preclinical experiment.

The cardioprotective effect was performed on isolated, modifies Langendorff-perfused rat hearts. The ischemia was induced by stopping of coronary flow followed by reperfusion. During a prophylactic administration flavonoids were applied orally. Biochemical indicators of oxidative damage in serum and myocardial tissue have been evaluated. The effects of both flavonoids on cardiac functions also were examined.

The results demonstrate that prophylactic administration of osajin and pomiferin attenuate the myocardial dysfunction provoked by ischemia-reperfusion.

The renoprotective effect was tested on model of unilateral ischemia-reperfusion of kidney tissue. During a prophylactic or therapeutic administration flavonoids were applied orally. Selected biochemical markers were assessed in blood. Renal functions were assessed too. The kidney tissue samples were used for histopathological examination.

The results obtained during prophylactic and therapeutic administration confirmed supposed effects of both substances.

The antidiabetic and antioxidative effect was monitored under the experimental conditions of alloxan-induced diabetes mellitus. During a therapeutic administration flavonoids were applied orally. Selected laboratory parameters in serum and in urine were determined. Kidney tissue and pancreas samples were taken for histopathological analysis.
The results of biochemical examination show a protective antioxidative and antidiabetic effect of both flavonoids. The results of histopathological examination correlate only partially with them.

P-6
The influence of chronic administration of thioacetamide on plasma levels of ALT, AST, GLDH and markers of oxidative stress in liver and kidneys of female wistar rats
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Thioacetamide (TAA) is a well known inducer of hepatic fibrosis and has been often used in experimental chronic hepatotoxicity models due to its ability to produce liver damage similar to human hepatic fibrosis. The highly specific hepatotoxic effect is mediated by thioacetamide-S,S-dioxide, the toxic product of TAA oxidation by hepatic microsomal enzyme CYP2E1. It is supposed that the generation of reactive oxygen species plays an important role in TAA induced liver damage and fibrosis. The aim of this preliminary experiment was to verify the time course of levels of ALT, AST and GLDH activities in plasma and markers of oxidative stress in liver and kidneys after chronic TAA administration.

Wistar albino female rats were divided into two groups: control and TAA-treated. TAA was applied intraperitoneally (200 mg/kg body weight) three times per week for a 12-week period. Animals in the control group received saline. One third of the animals of each group was sacrificed 4 weeks, one third 12 weeks and the remaining third 16 weeks after the end of the treatment period. Blood and tissue samples were collected. Lipid peroxidation (LP), reduced glutathione (GSH) and activities of glutathione peroxidase (GPx), glutathione reductase (GR) and catalase (CAT) were estimated in liver and kidney homogenates. ALT, AST and GLDH levels were determined in serum. TAA administration significantly increased ALT, AST and GLDH activities in plasma and markers of oxidative stress in liver and kidneys after the last TAA administration and catalase (in the 4th and the 12th week).

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P-7
Effect of developmental administration of antidepressant venlafaxine on selected behavioral variables in rat offspring
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The estimated prevalence of depression in pregnancy ranges from 9 to 16%. Treatment of depression in this critical period raises the question if to treat or not to treat depression during pregnancy. Consequences of both, the untreated depression or the antidepressant therapy represent a possible risk for injury of fetal and/or neonatal development. Selective serotonin re-uptake inhibitor (SSRI) and serotonin and noradrenaline re-uptake inhibitor (SNRI) drugs are commonly used for treatment of maternal depression. Venlafaxine belongs to the SNRI antidepressant drugs. The FDA has classified VENF regarding to pregnancy risk as C category of drug, which means that there are no well-controlled studies examining safety to the developing child. The aim of this study was to determine the effect of venlafaxine administration during sensitive functional brain development on neurobehavioral development of rat offspring. Pregnant Wistar rats were treated with venlafaxine (oral administration) from gestation day 15 to day 20 post partum (PP) at the doses of 7.5, 37.5 and 75 mg/kg. On day 4 PP all litters were subjected to „culling“ procedure (number of pups was reduced to 4 males and 4 females) and on day 21 PP they were weaned from their mothers. Animals were tested in an open field daily in 5 min sessions 5 consecutive days (22-26 PP), in an elevated plus maze (60 PP), in a light-dark box (80 PP) and in a forced swim test (85 PP) in individual 5 min sessions. Maternal VENF treatment resulted in a decreased intensity of locomotion in the offspring in the open field test and led to an increased activity in the forced swim test. In addition, we found reduced anxiety-like behavior in females compared to males exposed to VENF. The results suggest that VENF may gender-dependently interfere with functional development of the brain resulting in altered neurobehavioral regulations and adaptations in a new environment.
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P-8

Cyclin-dependent kinase inhibitors, AT-7519, DINACICLIB, PD 0332991, flavopiridol, and SNS-032, inhibit ABCB1 and ABCG2 transporters

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ABCB1 (P-glycoprotein) and ABCG2 (breast cancer resistance protein) are the most extensively studied drug efflux transporters. They contribute to multiple drug resistance in cancer and may be involved in the decreased anticancer efficiency and modified pharmacokinetic/pharmacodynamic properties of therapeutic entities. The cyclin dependent kinase inhibitors (CDKis), AT-7519, dinaciclib, PD 0332991, flavopiridol and SNS-032 are novel anticancer agents in phase I/II clinical trials for the treatment of various cancers and their influence on the function of membrane efflux transporters has not been described yet. The aim of the present study was to characterize the inhibitory effect of the selected CDKi, AT-7519, dinaciclib, PD 0332991, flavopiridol, and SNS-032, on ABCB1 and ABCG2 efflux activity. The intracellular daunorubicin accumulation in MDCKII-ABCB1 and mitoxantrone accumulation in MDCKII-ABCG2 cells was examined by flow cytometry. Parental MDCKII cells were analyzed as a control using both substrates separately. LY335979 or Ko143 were used as positive control for ABCB1 or ABCG2 inhibition, respectively. The increase in the intracellular accumulation of either daunorubicin or mitoxantrone reflects an inhibitory effect.

The intracellular accumulation of daunorubicin was increased 1.14, 1.49, 2.07, 3.73 and 5.04-fold in the presence of AT-7519, dinaciclib, SNS-032, flavopiridol and PD 0332991 at 50 μM concentration, respectively, in MDCKII-ABCB1 cells. The intracellular accumulation of mitoxantrone was increased 1.33, 1.91, 2.51, 4.23 and 4.79-fold in the presence of AT-7519, SNS-032, dinaciclib, flavopiridol and PD 0332991 at 50 μM concentration, respectively, in the MDCKII-ABCG2 cells. We observed that all CDKi inhibit ABCB1- or ABCG2-mediated efflux in a concentration dependent manner, but show no significant effects on the parental MDCKII cell line. We conclude that the inhibition of ABCB1 and/or ABCG2 transporters by the CDKi could affect the pharmacokinetic behavior of ABCB1/ABCG2 substrates if administered simultaneously.

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P-9

Incidence of thrombotic complications in patients with atrial fibrilation in anamnesis depending on the anticoagulant/antiaggregant therapy

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The atrial fibrilation represents one of the most common clinically indicated disorders of the cardiac dysrhythmia. Its increasing incidents correlates with increasing polymorbidity, age and sex of the patients. Nowadays, the number of patients in Czech Republic counts around 1% of the total Czech Republic population. A prevention of arterial complications represents an important aspect in treating the possible future worsening of a patient health state. The treatment is normally based on the anticoagulant or antiaggregant therapy.

The sample size involved 66 patients cured for six months in the Second Department of Surgery, St. Anne's University Hospital, Brno. Patients indicated with the increased risk of the arterial fibrillation were distributed into five groups (four groups according to the farmaco-therapy of the blood coagulation and one more group of patients for whom the anticoagulant/antiaggregant therapy has not been indicated). A correlation between all the five groups has been investigated with respect to the applied therapy and the incidence of the thromboembolic events.

In the full investigated ensemble of patients, the thromboembolic complications were indicated for 23 persons. In the group treated with warfarin, which in total involved 23 patients, seven persons were diagnosed with the arterial complications what represents 30.4%. In the second group, which in total included 29 persons treated with antiaggregation therapy by means of acetylsalicylic acid were 9 patients diagnosed with the thromboembolic complication. In this group, the relative number of impacts represents 31%. Finally, it has been found that in the group with no anti-coagulant therapy the risk of the thromboembolic events increased up to 70%.

The obtained results suggest that the risk of the complications is similar in both groups treated by either warfarin or acetylsalicylic acid, which are both common drugs applied in the situations where blood coagulation is an issue. While in the most cases that, in spite of the warfarin
treatment, lead to the complications, the reason of the unsuccessful therapy could be associated with the non-optimal level of INR. Moreover, we cannot also exclude a high rate of the drug therapy interactions as a reason of the anticoagulant or antiaggregant failure.

P-10
Impact of diabetic state in patients with end-stage heart failure and its association with cardiac expression of microRNAs

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Introduction. Diabetes mellitus is known cardiovascular disease risk factor and co-morbidity in heart failure, linked with progressive end-organ damage. High or uncontrolled blood sugar may affect the expression of small regulatory RNAs - microRNAs, which are fine tuners of multiple aspects of structure and function of the heart as revealed by bioinformatic predictions. The aim of this study is to determine possible associations or differences between diabetic and nondiabetic patients with end-stage heart failure on the level of various clinical parameters and myocardial microRNAs.

Methods. Cohort consists of 41 patients with end-stage heart failure (14 diabetic, 27 non-diabetic), who underwent heart transplantation. From every patient, we collected a set of clinical parameters: age, sex, underlying cause of heart failure, BMI, blood pressure, analyzed are also values of ECG, ECHO, cardiac catheterization and serum biochemistry. We assessed the expression of microRNAs relevant for heart (miR-1, miR-133a, miR-208a, miR-208b, miR-499, miR-29b) by modified real-time PCR in explanted left ventricular samples.

Results. Diabetic patients had clearly higher blood sugar (+36%), but also serum levels of urea (+48%) and uric acid (+36%), as well as body mass index (BMI; +13%). No other of 26 remaining various parameters significantly differed between diabetic and nondiabetic population of patients with failing hearts. The same is true also for analyzed cardiac microRNAs (miR-1, miR-133a, miR-208a, miR-208b, miR-499, miR-29b) by modified real-time PCR in explanted left ventricular samples.

Conclusion. This study was based on hypothesis, that higher or uncontrolled glyemia in patients with failing hearts may result in changed molecular frame of the myocardium and thus in worsened prognosis. Contrary to this hypothesis, diabetic patients didn’t exhibit differences in expression of analyzed cardiac microRNAs from non-diabetic patients. Conclusively, worsened prognosis for diabetic patients may be more likely the result of damage to other organ than the heart itself.

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P-11
Alkaloid boldine is a ligand of farnesoid x receptor

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Boldine is the major alkaloid from Chilean boldo tree, and is used in traditional medicine to support bile production, but the scientific data for this effect are lacking. Recently we found immediate choleretic activity of boldine in rats, which depended on actual concentration of the agent in the bile.

In the current study, we analyzed whether boldine activates Farnesoid X receptor (FXR), which controls key genes involved in bile acids synthesis, transport and their homeostasis in the body. We used gene reporter assays with pFXRE-luc2P reporter construct and the fusion expression construct containing the ligand binding domains of human FXR receptor fused to GAL4 to analyze interaction of boldine with human FXR. Experiments have been performed in HepG2 cells with the constructs treated for 24 h with increasing concentrations of boldine alone or in combination with chenodeoxycholic acid (CDCA) as a FXR ligand. We found interaction of boldine with FXR in both experimental systems. We can therefore conclude that boldine may be a weak ligand of FXR receptor, which correlates with its choleretic effect in rats.

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P-12

Participation of opioid receptors in the antitussive activity of Withania somnifera

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In last decades treatment by plant substances has become one of the leading forms of treatment many diseases of the respiratory system, including cough. Therefore, the subject of our interest became plant polysaccharide isolated from Withania somnifera and its antitussive activity. Except of that, we tried to find out what role opioid receptors play in that activity.

The experiments were carried out on the conscious male guinea pigs (Trik). The plant substance (Withania somnifera) was apllied to the first group of guinea pigs perorally. The cough reflex was evoked by citric acid in concentration 0.3 M. To second group of guinea pigs, we applied 15 min before own application of arabinogalactan (WS), Naloxon hydrochlorid (i.p.). Nonselective antagonist Naloxon methiodid was apllied (15 min before WS) to the third group of animals.

Polysaccharide isolated from WS showed the ability after oral administration reduced the parameters of citric acid induced cough in awaken guinea pigs healthy in vivo experimental conditions. We showed that on reduction of cough reflex induced by administration of arabinogalactan from Withania somnifera are participated both centrally and peripherally acting opioid receptors.

Our results supported our previous findings that naturally occurring polysaccharides possess antitussive activity. This study also represents the participation of centrally and peripherally acting opioid receptors on reduction of cough reflex induced by Withania somnifera.

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P-13

Three years of experience of the attention hyperactivity disorder (ADHD) treatment with atomoxetine and methylphenidate

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Attention hyperactivity disorder (ADHD) is the most frequent psychiatric behavioural disorder in school children (8-10%). Retrospectively (2010-2013), we analysed 50 patients (82/18 %, M/F), with ADHD. Average age was 11.9 ±0.4, (5-18 years). Together 48 % of the children were from foster homes. The most frequent comorbidities were: F.90 - disturbance of activity and attention (84%), F.80 - specific developmental disorders of speech and language (36%) and F.92 - mixed disorders of conduct and emotions (36 %). For the classification of the comorbidities we used ICD 10. In the pharmacotherapy we were analysing ATC class, indication, dosing in paediatric indication). As information source about the drugs we used the official web side of State Institute for Drug Control. All children were treated with sympathomimetics atomoxetine (80%) or methylfenidate (52%). The second most present group were antipsychotics (90%, risperidone/thiapridal 3:2), and third one antiasthmatics (66%, promethazine/cyproheptadine 2:1). Out of the most frequently used drugs thiapridal was used off label. Because of high prevalence of several comorbidities, combined therapy prevailed; most patients (38%) took 4 drugs. Potential adverse event was defined as coincidence of following factors: presence of subjective discomfort, presence of this discomfort in the Summary of product characteristics (SmPC) and intervention in the therapy. Potential adverse events were present in 48% of the patients. Together 50% of the patients who were treated with atomoxetine had potential adverse events, in methylphenidate treated patients it was 15.4% of the patients. Most frequent adverse event was anorexia (27%/11.5% of the atomoxetine/ methylphenidate patients). This the physician solved by adding cyproheptadine into the treatment. Hyperkinetic disorder is complex, multifactorial disease. Although the pharmacological possibilities, the success of the treatment depends on the cooperation of multidisciplinary team of professionals and parents.
P-14

Long-term consumption of Coca-Cola results in development of manifestations of metabolic syndrome in Wistar rats

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**Background.** In rat, the use of Coca cola to induce an experimental metabolic syndrome has not been clarified yet. We aimed to describe the effect of long-term (three months) consumption of Coca cola on development of metabolic syndrome in rat.

**Methods.** We used 10 weeks old male Wistar rats receiving a standard diet. Additionally, a group of rats received a commercially available Coca-Cola beverage (CC, n=13), controls (CON, n=7) drank common drinking water. We measured weight gain, and plasma glucose (Glc) and cholesterol (Chol) levels by using standard glucometer in capillary blood drops. In addition, systolic and diastolic blood pressures (sBP and dBP) and heart rate (HR) were measured by using tail-cuff method.

**Results.** We observed a significantly increased body weight in CC rats (391±12 g; \( P<0.01 \)) when compared to controls (342±19 g). This was in accordance with significantly increased sBP (CC: 141±4 mmHg vs. CON: 119±7 mmHg; \( P<0.01 \)) and a tendency of increased dBP (CC: 98±2 mmHg vs. CON: 87±6 mmHg; NS) and significantly increased HR (CC: 361±8 mmHg vs. CON: 310±15 mmHg; \( P<0.01 \)). Additionally, we measured a tendency of increased Glc (CC: 5.65±0.39 mmol/L vs. 4.77±0.51 mmol/L; NS) but stable cholesterol Chol (CC: 4.10±0.06 mmol/L vs. CON: 4.21±0.07 mmol/L; NS).

**Conclusion.** Long-term administration of Coca-Cola can lead to development of cardiovascular (increased BP and HR) as well as metabolic manifestations (impaired glucose homeostasis) of metabolic syndrome in Wistar rat. Consequently, we propose this model as a reliable experimental model of rat metabolic syndrome.

ACKNOWLEDGMENT

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P-15

The role of ghrelin and orexin systems participation in fear and anxiety behavior testing, using aversive ultrasonic vocalization in rats

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**Aims.** Orexigenic peptides ghrelin and orexin exhibit central neurobiological effects which are involved in food intake, reward, learning, memory, cognition and mood. Both peptides elicit mostly pro-anxiety effects. Thus we wanted to test the potential anxiolytic effects of the ghrelin receptor antagonist (JM2959) and orexin receptor antagonist (SB334867) which has yet to be done and for the first time use the aversive ultrasonic vocalization in rats for testing these peptides mechanisms participation.

**Methods.** One day before the experiment the aversive behavior was established (i.e. rats were individually exposed in the automatic chamber for 7 min to 6 electro-shocks). The aversion was fixed on the next day (1 shock during 1 min exposure). After which ghrelin (30; 300 μg/kg) or antagonist (JM2959 - 0.6, 6; 12 mg/kg, SB334867 – 1; 10; 20 mg/kg) or saline was i.p. applied and 30 min later the rat was placed into the chamber again for 10 min (no electro-shocks) and its ultrasonic aversive vocalization was automatically measured. The anxiolytic effect was considered compared to the control/saline rats. The ghrelin antagonist significantly decreased aversive ultrasonic vocalization in doses 6 mg/kg (decrease of 74.2%) and 12 mg/kg (decrease of 62.8%) versus control. The observed SB33487-induced anxiolytic-like and ghrelin-induced anxiety-like effects were not significant.

**Results.** We have found that ghrelin antagonist significantly decreased the rats aversive ultrasonic vocalization, which supports the view, that ghrelin antagonism could be used for the prevention of distress and anxiety-induced overeating and drug consumption.

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This study was supported by IGA NT/13687-3/2012, 266705/SVV/2013 and PRVOUK P34.
**P-16**

**Resistance of cancer cells to anthracyclines mediated by reductive metabolism: the role of aldo-keto reductase 1C3**

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Drug resistance belongs to the main obstacles emerging during the cancer chemotherapy. By the conversion of drugs into corresponding less active metabolites, some biotransformation enzymes significantly contribute to this phenomenon. In this study, we investigate possible role of aldo-keto reductase 1C3 (AKR1C3) in the resistance of cancer cells to anthracyclines. Reducing activity of AKR1C3 toward anthracyclines was first tested using incubations with purified recombinant enzyme prepared in *E. Coli*. AKR1C3 was shown to most efficiently catalyse the formation of daunorubicinol, followed by idarubicinol and doxorubicinol. We further examined the reduction of daunorubicin and idarubicin employing transfection of A549, HeLa, MCF7 and HCT 116 cancer cells with AKR1C3 encoding vector. Production of daunorubicinol and idarubicinol was greatly accelerated in transfected cells; this acceleration was significantly blocked by 2'-hydroxyflavanone, a recognised AKR1C3 inhibitor. To demonstrate the participation of AKR1C3 on anthracycline resistance, we performed MTT proliferation assays employing transfected HeLa, MCF7 and HCT 116 cells. Introduction of AKR1C3 into cells significantly reduced the antiproliferative effect of both daunorubicin and idarubicin in all three tested cell lines. In conclusion, our data suggest substantial impact of AKR1C3 on the pharmacokinetic behaviour of daunorubicin and idarubicin. In addition, we demonstrate that the reduction of daunorubicin and idarubicin catalysed by AKR1C3 contributes to the resistance of cancer cells to anthracycline chemotherapy. Our results should be considered in the clinical use of these anthracyclines.

**ACKNOWLEDGEMENT**

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**P-17**

**Comparison of methylphenidate use in patients with ADHD hospitalized in clinic of psychiatry, Jessenius Medical Faculty, Comenius University between years 2009-2012**

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The current study investigated methylphenidate (MPH) use and its efficacy in treating ADHD in children and adolescent patients hospitalized in Clinic of Psychiatry JFMED CU between the years 2009-2012. We aimed in use of other most prevalent therapeutic strategies and most prevalent comorbid disorders accompanying ADHD. Efficiency of therapy was evaluated by Clinical Global Impression Scale (CGI), Severity (S) and Improvement (I) subscale. The study aimed also in gender and age differences in groups of patients. This retrospective, observational and comparative study evaluate use of MPH in hospitalized patients during 4 years (2009-2012) from the time when the stimulants were registered in SR. Data were obtained from medical records of hospitalized patients, aged 6-17 years. The study included 104 hospitalizations, 77 patients treated with MPH. Statistical methods used in this study were: statistical analyses, comparative and correlation methods. There is no significant difference in gender and selected period in ADHD patients treated by MPH - the proportion of girls and boys is stable over time. The most frequent disorders accompanying ADHD were Conduct disorders (51%), Emotional disorders (16.6%) and other specified behavioural and emotional disorders (12.6%). There is no difference in severity of ADHD of incoming patients the most common CGI severity grade is 5 (markedly ill). There is no significant correlation between ADHD CGI-S and CGI-I. The most common CGI-I grade was 2 (much improved), scored in 44.6 % of all inpatients. In the period 2009-2010 mono-therapy by MPH prevailed, in the period 2011-2012 we recorded the trend of a combination of MPH with another psycho-pharmacotherapy. We found significant difference in use of combination of MPH with another drug therapy between years 2009-2012 (P=0.020). The most often used psychopharmacology were antipsychotics levomethadone (21.2%), chlorpromazine (12.12%) and antihistamine promethazine (12.12%). We did not find a significant relationship between the CGI-I grade of behavioural disturbances and the use of mono-therapy or combination therapy in patients with ADHD and conduct disorder.
Organic cation transporter 1 (OCT1, SLC22A1) is a membrane transporter that is important for uptake of numerous cationic drugs into hepatocytes. Its liver-specific expression in hepatocytes is strongly controlled by hepatocyte nuclear factor-4α (HNF4α). Recently we found that dexamethasone through glucocorticoid receptor (GR) significantly up-regulates OCT1 mRNA and protein in primary human hepatocytes. Therefore we examined direct (GR response element-mediated) and indirect transactivation of OCT1 gene in primary human hepatocytes. We also examined which other liver-enriched transcription factors are involved in OCT1 gene expression and whether they are regulated by dexamethasone. Notably, expression of major nuclear receptors HNF4α, Pregnane X receptor, Retinoid X receptor and Constitutive androstane receptor (CAR) have been demonstrated to be augmented by glucocorticoid receptor in human liver.

Gene reporter construct with 2 kb promoter sequence of the human OCT1 gene was not responsive to glucocorticoids in cells cotransfected with GR expression construct, but was sensitive to CCAAT/enhancer binding proteins β (C/EBPβ) and HNF4α cotransfection in HepG2 cells. Viral transduction of MZ-Hep1 cells with the expression constructs for HNF4α, CCAAT/enhancer binding proteins β (C/EBPβ) and peroxisome proliferator-activated receptor γ coactivator 1α (PGC1α), but not with PXR nor CAR, demonstrated significant roles of the transcription factors in OCT1 gene induction. Consistently, we found that expression of OCT1 mRNA in human livers significantly correlates with C/EBPβ and HNF4α mRNAs expression. We observed that HNF4α is induced by dexamethasone in primary human hepatocytes, but not in hepatocyte-derived cell lines. However, neither C/EBPβ nor PGC1α were up-regulated in human hepatocytes by dexamethasone.

We can conclude that OCT1 is induced only through GR nuclear receptor via an up-regulation of HNF4α in primary human hepatocytes.

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average duration of the treatment was 6.7 years. Adverse effects were observed in 18.5% of women, mostly nausea, weight gain, cephalaea and vertigo.

Although there are concerns about adverse effects, HRT still plays a significant role in the treatment of menopausal symptoms. Risks can be decreased and benefits maximized by choice of optimal and individually-tailored therapeutic regimen.

**P-20**
The effectiveness of additional antioxidant to antidiabetic therapy in diabetic - hypertensive rats

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Hypertension during diabetes is a risk factor for development of endothelial dysfunction. The aim of this work was to evaluate the effect of combination therapy of diabetic hypertensive rats with antioxidant Pycnogenol and antidiabetic drug metformin drugs on improvement of endothelial dysfunction assessed as the reactivity of the vessels.

Diabetes was induced in the spontaneous hypertensive rats (SHR) by intraperitoneal injection of streptozotocin (3 x 25 mg/kg/b.w). The treatment with Pycnogenol, metformin or their combination lasted for 6 weeks. After the treatment, the animals were sacrificed and vascular contraction and relaxation responses of the isolated aorta were registered by SPEL Advanced ISOSYS system.

The relaxation responses in untreated diabetic and diabetic - hypertensive animals were significantly decreased compared to healthy control animals (D 55.36% ± 7.65% / HD 55.54% ± 9.07% / C: 80.61% ± 3.04%, P<0.05). The relaxation responses of animals treated with Pycnogenol, metformin or their combination showed increased vascular relaxation response compared to untreated animals. Pycnogenol monotherapy led to a significantly highest improvement of vascular relaxation response (HD-P 129.26% ± 8.88%, P<0.001). Metformin itself and its combination with Pycnogenol also resulted in a significant increase in relaxation of blood vessels compared to untreated diabetic - hypertensive animals (HD-M 111.38% ± 7.28%, P<0.01 / HD-PM 108.33% ± 14.45%, P<0.05).

The therapy with Pycnogenol, metformin and their combination improved vascular relaxation responses that suggest improvement of endothelial dysfunction as well. However the effectiveness of additional antioxidant to antidiabetic therapy in diabetic - hypertensive rats was confirmed to be surprisingly lower than monotherapy.

**ACKNOWLEDGMENT**

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**P-21**
Pharmacokinetics of dexrazoxane and its putative active metabolite in rabbits in relationship to the cardioprotective effects of the drug

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Pycnogenol, metformin and their combination lasted for 6 weeks. After the treatment, the animals were sacrificed by Kinetica 4.0 software. We found mean plasma c_{max} (406 and 51 μM), t_{max} (0.22 and 2.2 h) for DEX and ADR-925, respectively and mean t_{1/2} (DEX) 2.0 h. AUC 0-6h (DEX/ADR-925) ratios were 2.9 and 3.4.

In the myocardium, the selected parameters for DEX and ADR were as follows: c_{max} (207 and 14 pmol/mg wet weight), t_{max} (0.5 and 12 h) and AUC_{0-12h} ratio was 5.3. Furthermore, more complex insight was obtained using population-based PK approach and the data are being compared to pharmacodynamic parameters.
P-22

Cilia in the respiratory tract during experimental conditions

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In addition to the airway defense reflexes, mucociliary transport is responsible for the protection of the airways and lungs against excess of mucus and foreign particles. Cilium is efficient component of this primary innate defense mechanism.

The purpose of this study was to make the model for analysis of ciliary beat frequency in the airway epithelial cells and evaluate the role of cholinergic receptors in the ciliary beating during \textit{in vitro} conditions.

The experiment was performed on the tracheal ciliated cells of freshly excised mucosa. The trachea of guinea pigs was used. Next microscopic sample examination using light microscope, and high speed camera connected with computer was carried out. Short videos were recorded and assessed by ciliary analysis software and following postanalysis. The ciliary beat frequency was determined to describe the ciliary beating. Cholinergic response to metacholine (10\textsuperscript{-8} mol.L\textsuperscript{-1}) and metacholine (10\textsuperscript{-8} mol.L\textsuperscript{-1}) plus atropine (10\textsuperscript{-8} mol.L\textsuperscript{-1}) was monitored after their addition to ciliated cells.

The experimental model for studying of ciliary kinetics in which cholinergic system plays a role in the modulation of ciliary beating was created by us.

ACKNOWLEDGEMENT

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P-23

The evaluation of the reactivating and therapeutic efficacy of two novel bispyridinium oximes (K361, K378) in comparison with the oxime K203 and trimedoxime in tabun-poisoned rats and mice

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The potency of two newly developed bispyridinium compounds (K361, K378) to reactivate tabun-inhibited acetylcholinesterase and reduce acute toxicity of tabun was compared with the oxime K203 and trimedoxime using \textit{in vivo} methods. The study determining percentage of reactivation of tabun-inhibited diaphragm acetylcholinesterase in poisoned rats showed that the reactivating efficacy of the oxime K378 is slightly lower than the reactivating potency of the oxime K203 and trimedoxime while the ability of the oxime K361 to reactivate tabun-inhibited acetylcholinesterase is markedly lower compared to the oxime K203 and trimedoxime. In the brain, the potency of both newly developed oximes to reactivate tabun-inhibited acetylcholinesterase was negligible. The therapeutic efficacy of both newly developed oximes corresponds to their weak reactivating efficacy. Their potency to reduce acute toxicity of tabun was significantly lower compared to the oxime K203 as well as trimedoxime. In conclusion, the reactivating and therapeutic potency of both newly developed oximes does not prevail the effectiveness of the oxime K203 and trimedoxime and, therefore, they are not suitable for their replacement of commonly used oximes for the treatment of acute tabun poisoning.

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P-24

Cardiomarkers in isoproterenol–induced model of cardiotoxicity in rats

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Troponin is a protein, which plays an important role in the muscle contraction. During myocardial ischemia,
its cardiospecific isoforms (cTnI and cTnT) are released from cardiomyocytes into blood where they are determined. Nowadays, troponin I and T are widely used in clinical medicine as a marker of acute coronary syndrome.

This pilot study focused on the use of cardiomarkers in preclinical testing, where they can help to improve primary screening of adverse effects and detect possible cardiotoxicity of newly synthesized drugs. We chose troponin I as a marker due to its high sensitivity and specificity, and added two other markers (CK-MB and NT-proBNP) for comparison.

We induced a model of acute cardiotoxicity by isoprenaline hydrochloride (ISO) in toxic doses. We investigated a group of 15 male Wistar rats, which was divided into four subgroups differing in the way of administration (i.v., i.p. or s.c.) and doses of isoprenaline provided by literary sources. Three subgroups were experimental and they were administered isoprenaline, one subgroup was used as a control. We collected blood samples at predetermined intervals, centrifuged them and stored them at -80°C until the final determination. Cardiomarkers (cTnI, CK-MB and NT-proBNP) were assessed in each plasma sample using the Dimension ExL analyzer with LM Integrated Chemistry System- Siemens® in the laboratory department of Trauma Hospital of Brno.

The most important finding was the positive outcome, which means the possibility of using human kits for the detection of cTnI and CK-MB in animals. It confirms the fact that the structure of troponin is highly conserved across species. Unfortunately, it was impossible to measure NT-proBNP by the device due to unknown reason. The best way of administration, from the perspective of simplicity of application and low mortality, was the intraperitoneal application of ISO at the dose 50 mg/kg b.w. The concentration of cTnI and CK-MB in i.p. group had an increasing tendency. Troponin showed an early onset of the release and the curve line describing its concentration dependent on time dropped already after 6 h. Creatine kinase showed an increasing concentration till the end of our monitoring (24 h). Concentration of markers in the control group was also detectable but in comparison with experimental groups, values of markers in the control group were always lower.

The aim of the open-label, single dose, randomized, crossover (2-sequence, 2-period), clinical study was to compare bioavailability of two oral tablet molsidomine (NO-donor used for the management of angina pectoris) formulations (A and B).

Twenty four healthy volunteers (12 men and 12 women, 18-51 years, body mass index 19.1-31.1 kg/m²) received twice a single 4 mg oral dose of molsidomine with a washout period of 7-14 days. Blood samples (10 mL) were taken 0-10 h after drug administration for the measurement of plasma concentrations of molsidomine (using HPLC method with UV detection). Pharmacokinetic parameters were estimated using non-compartmental methods. For their statistical evaluation, ANOVA, two one-sided tests procedure and estimations of 90% confidence intervals (90% CI) were used.

The results of the study are presented in the following table:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Formulation A</th>
<th>Formulation B</th>
<th>Point estimate</th>
<th>90% CI</th>
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</thead>
<tbody>
<tr>
<td>AUC₀₋ₐ₈₀ [ng.h/ml]</td>
<td>120 ± 65</td>
<td>111 ± 58</td>
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<tr>
<td>AUC₀₋₈₀ [ng.h/ml]</td>
<td>110 ± 60</td>
<td>101 ± 54</td>
<td>110.5 %</td>
<td>98.2 – 124.4 %</td>
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<tr>
<td>AUC₀₋₅₀ [ng.h/ml]</td>
<td>111 ± 60</td>
<td>102 ± 54</td>
<td>110.6 %</td>
<td>98.3 – 124.4 %</td>
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<tr>
<td>Cₘₐₓ [ng/ml]</td>
<td>42.5 ± 15.4</td>
<td>43.2 ± 20.5</td>
<td>101.0 %</td>
<td>92.0 – 110.8 %</td>
</tr>
<tr>
<td>tₘₐₓ [min]</td>
<td>33 ± 18</td>
<td>40 ± 23</td>
<td>-10 min</td>
<td>-15 to 0 min</td>
</tr>
<tr>
<td>kₑ [h⁻¹]</td>
<td>0.419 ± 0.100</td>
<td>0.435 ± 0.132</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>t₁/₂ [h]</td>
<td>1.76 ± 0.50</td>
<td>1.75 ± 0.59</td>
<td>-</td>
<td>-</td>
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</table>

Bioequivalence of the two compared molsidomine formulations was proven for all bioavailability parameters (in the acceptance range 80-125%).
Inhibition of P450 activities by anthocyanidins and anthocyanins

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Anthocyanins are a water soluble natural pigments which belong to a large group of polyphenolic named flavonoids. They are widely distributed in fruit and vegetable such as bilberries, cranberries, strawberries, grapes, and red cabbage but are also found in flowers and other plant materials. Anthocyanins are responsible for cyanic colors ranging from salmon pink through red and violet to dark blue. There have been over 600 anthocyanins identified in nature, featuring six common aglycones – anthocyanidins (cyanidin, delphinidin, malvidin, peonidin, petunidin and pelargonidin) and various types of glycosylations. Anthocyanins possess anti-inflammatory and anti-carcinogenic activity, cardiovascular disease prevention, all of which are associated with their potent antioxidant property. Interaction of three forms of human hepatic cytochromes P450 - CYP3A4 (testosterone 6β-hydroxylation), CYP1A2 (7-ethoxyresorufin O-deethylation) and CYP2C9 (diclofenac 4'-hydroxylation) with six anthocyanidins (cyanidin, delphinidin, malvidin, peonidin, petunidin and pelargonidin) and nine anthocyanins (cyanidin-3-glucoside, cyanidin-3-galactoside, cyanidin-3-arabinoside, cyanidin-3,5-di-glucoside, delphinidin-3-glucoside, malvidin-3-glucoside, peonidin-3-glucoside, petunidin-3-glucoside and pelargonidin-3-rutinoside) were studied using pooled human microsomes. The most influenced activities were interactions of CYP3A4 with delphinidin, petunidin, peonidin and pelargonidin, CYP1A2 with delphinidin and CYP2C9 with pelargonidin, peonidin, malvidin and peonidin.

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Lipolytic effects of newly synthesized aryloxypropanolamine derivatives in vitro

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Adipose tissue is a highly metabolically active organ with endocrine/paracrine function involved in regulation of various biological processes including energy metabolism, neuroendocrine and immune processes. Excess of adipose tissue is related to obesity, insulin resistance and metabolic syndrome development, which is reaching the epidemic proportions and is the main cause of death in western countries. The use of β-adrenergic receptor agonists represents one of the potential pharmacological approach in metabolic syndrome treatment.

In this study, the lipolytic activity of twelve newly synthesized aryloxypropanolamine derivatives as potential β3-agonists were tested using cultured human subcutaneous adipocytes and was compared with Isoproterenol and BRL-37344 as positive controls. The tested homologous series substances differ in substituents on the basic skeleton and were synthesized at Department of Chemical Drugs of Pharmaceutical Faculty VFU Brno. The cultured human subcutaneous preadipocytes Zen-Bio, Inc. were allowed to differentiate for one week under standard differentiation conditions at 37 °C in 5% CO₂ atmosphere. The tested substances, isoproterenol and BRL-37344 (Sigma), were added at a concentration of 0.0075μmol/L and incubated at 37 °C for 3 h. Lipolytic activity was assessed as non-esterified fatty acids (NEFA) released by adipocytes. The concentration of NEFA was determined by spectrophotometry at 540 nm.

In four substances the rate of lipolysis was higher than in positive control - isoproterenol , in four substances there were no differences compared to positive control - BRL-37344 observed and in four substances lipolysis was not affected.
P-28

The influence of oxidative stress markers in diabetic and hypertensive-diabetic rats by combination of Pycnogenol® and metformin

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The oxidative stress formed in connection with hyperglycaemia has considerable effect in hypertension development in terms of diabetes. An increase of free radicals concentration supports lipid peroxidation and insulin resistance. The aim of work was to evaluate MDA and NAGA level changes in control, diabetic and hypertensive-diabetic rats after administration of Pycnogenol®, metformin and their combination. Wistar male rats and spontaneously hypertensive rats were randomly divided into 9 groups. Diabetes was induced in 8 groups by intraperitoneal application of streptozotocin (25 mg/kg, i. p.) during three consequent days. In the last group there were Wistar male rats without diabetes (Control). We started administration with Pycnogenol®, metformin and their combination one week later. Levels of MDA in liver and NAGA in kidney were evaluated after 6 weeks of therapy. The levels of MDA in liver were increased only in diabetic animals compared to control. All substances decreased the levels of MDA in diabetic (D) and hypertensive–diabetic (HD) group compared to animals without treatment. The most effectively treatment was metformin in both pathological groups. In contrast to monotherapy the combination of these drugs was the less effective in lowering of MDA levels in D and HD animals. The specific activity of NAGA in kidney was significantly increased only in HD group compared to control and D group. In contrast to D rats without treatment the following substances did not influence the NAGA levels in kidney in monotherapy and in their combination. On the other hand both substances decreased the NAGA levels in kidney of HD rats compared to HD rats without treatment; the most significantly Pycnogenol®. The decrease of MDA level and NAGA activity was less effective in groups where the combination of treatment was applied compared to the other treated animal groups. Diabetic animal groups responded to the therapy better than diabetic-hypertensive animal groups.

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P-29

Paroxetine blocks reinstatement of methamphetamine seeking behaviour in an animal model of depression

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The incidence of methamphetamine (METH) addiction among individuals with generalised depressive disorder is very high in accordance with the self-medication hypothesis suggesting that symptoms of depression may be relieved by the aminergic drugs of abuse. We developed an animal model of this comorbidity (IV METH self-administration in olfactory bulbectomy model of depression in rats) in which we recorded a higher METH intake and relapse rate. The anticipation for the present study was that the treatment of bulbectomized rats (OBX) with antidepressant paroxetine might normalise increased METH intake and relapse tendency.

Adult male Wistar rats were randomly divided into 2 groups and bilateral OBX/sham (SH) surgery were performed. The model of methamphetamine IVSA was initiated under fixed ratio schedule of reinforcement using operant chambers. After reaching a stable METH intake, forced abstinence (no access to the drug) and chronic paroxetine (PRX, dose: 10 mg/kg IP) or vehicle (VEH, dose: 3 ml/kg IP, saline) treatment was initiated. After 14 days one reinstatement session was performed. Thus, there were 4 groups of animals: SH VEH (n=6), SH PRX (n=5), OBX VEH (n=6), OBX PRX (n=5). Statistics: parametric one-way analysis of variance and paired t-test.

The OBX animals exhibited significantly higher tendency to relapse in terms of METH intake and active nosepoke responding. Chronic treatment with paroxetine abolished higher METH intake in the reinstatement session. In sham operated animals paroxetine did not show significant effects.

These results indicate that paroxetine is able to normalize some of the characteristics of the OBX model. Serotonin is known to potently modulate dopamine levels in the reward pathway and inhibition of serotonin transporters was reported to decrease methamphetamine craving. Therefore, investigation of specific serotonergic drugs might be an important step towards finding a novel treatment of drug addiction and relapse prevention.
Primary depression belongs to the most common causes leading to psychiatric hospitalization. Tendency of prescription of broader receptor-neurotransmission profile substances rises in recent years, utilizing a combination of antidepressants (AD), or antidepressants and antipsychotics (AP) in many cases. Prevalence of the therapeutic strategies and comorbid disorders accompanying depression were compared in retrospective, observational study. Medical records of all patients older than 18 years, hospitalized at Clinic of Psychiatry JFM CU Martin were studied and statistically analyzed in 5 years period. Significantly different gender distributions were witnessed in 2008 when compared with 2012. Significantly different presentations of solitary and recurrent depression in the years 2008 and 2012 with predominant single episodes of depression in 2012 and a prevalence of recurrent depression in 2008 were observed. Significant differences in the degree of depression (acc. to ICD-10) with more severe degrees of depression in 2012 were found. However, initial disease severity (CGI-S) showed no significant differences between the two compared years. There were significant differences in the rate of improvement at the end of hospitalization (CGI-I) with a marked improvement in 2008 (1.73 ± 0.65 vs. 1.91 ± 0.64). A significant increase in co-morbidities was witnessed in 2012. There was no significant correlation between severity of disease at the start of hospitalization and rate of improvement at the end of stay. No significant differences were evident in the use of venlafaxine and escitalopram. A significant rise in agomelatine prescriptions was noted in 2012. The most common antidepressants prescribed during the study were escitalopram, venlafaxine, and trazodone. The 2nd generation atypical antipsychotic co-medication was significantly increased in 2012 (52 vs. 68%, P=0.012), of which sulpiride experienced the most prominent growth. The most commonly prescribed 2nd generation AP was olanzapine. Decrease in 1st generation antipsychotics prescription was seen in 2012 and only slight increases were observed in prescription of anxiolytics and mood stabilizers in 2012.

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P-30
Depression treatment comparison of adult patients hospitalized in year 2008 and 2012 at Clinic of Psychiatry, Jessenius Faculty of Medicine, Comenius University, Martin

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Primary depression belongs to the most common causes leading to psychiatric hospitalization. Tendency of prescription of broader receptor-neurotransmission profile substances rises in recent years, utilizing a combination of antidepressants (AD), or antidepressants and antipsychotics (AP) in many cases. Prevalence of the therapeutic strategies and comorbid disorders accompanying depression were compared in retrospective, observational study. Medical records of all patients older than 18 years, hospitalized at Clinic of Psychiatry JFM CU Martin were studied and statistically analyzed in 5 years period. Significantly different gender distributions were witnessed in 2008 when compared with 2012. Significantly different presentations of solitary and recurrent depression in the years 2008 and 2012 with predominant single episodes of depression in 2012 and a prevalence of recurrent depression in 2008 were observed. Significant differences in the degree of depression (acc. to ICD-10) with more severe degrees of depression in 2012 were found. However, initial disease severity (CGI-S) showed no significant differences between the two compared years. There were significant differences in the rate of improvement at the end of hospitalization (CGI-I) with a marked improvement in 2008 (1.73 ± 0.65 vs. 1.91 ± 0.64). A significant increase in co-morbidities was witnessed in 2012. There was no significant correlation between severity of disease at the start of hospitalization and rate of improvement at the end of stay. No significant differences were evident in the use of venlafaxine and escitalopram. A significant rise in agomelatine prescriptions was noted in 2012. The most common antidepressants prescribed during the study were escitalopram, venlafaxine, and trazodone. The 2nd generation atypical antipsychotic co-medication was significantly increased in 2012 (52 vs. 68%, P=0.012), of which sulpiride experienced the most prominent growth. The most commonly prescribed 2nd generation AP was olanzapine. Decrease in 1st generation antipsychotics prescription was seen in 2012 and only slight increases were observed in prescription of anxiolytics and mood stabilizers in 2012.

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P-31
Effect of different doses of atropine on gastric myoelectric activity in experimental pigs

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Surface electrogastrography (EGG) is a non-invasive method for clinical assessment of gastric myoelectric activity. Our group has demonstrated that EGG is also reliable and feasible in experimental pigs. Porcine EGG is fully comparable with that recorded in healthy humans.

The aim of this project was to evaluate the effect of different doses of atropine on the gastric myoelectric activity in experimental pigs. Six mature young female experimental pigs (Sus scrofa f. domestica, mean weight 27±1.5 kg) entered the study three-times within three weeks.

A baseline EGG recording lasted 15 min Intramuscular atropine 1.5 mg (part A) or 3.0 mg (part B) or 4.5 mg (part C) was administrated. A total of seven 15-min intervals were recorded afterwards. Surface cutaneous EGG was recorded under general anaesthesia using an Electrogastrography System (MMS, Enschede, the Netherlands). Running spectral analysis (based on Fourier transform) was used for the elemental evaluation of the EGG. The results were expressed as running spectrum percent activity and the dominant frequency of slow waves was set at all intervals of EGG recordings. In part A, dominant frequency of slow waves increased from the basal values (3.0±0.6 cycles per min) to the maximum at 60 min (3.2±0.4) and at 105 min (3.2±0.3), both P<0.001. In part B, dominant frequency of slow waves increased from the basal values (2.7±0.5) to the maximum at 15 min (3.0±0.5) and at 75 to 105 min intervals (3.2±0.7 cycles per min), all P<0.001. In part C, there was a decrease not significant from the basal values (3.2±0.6) to the minimum at 75 min. (3.0±0.5 cycles per min.). Other changes of the dominant frequency were low. Heart rate...
increased after atropine administration in the part A from basal 74±4 beats per min. to the maximum at 65 min (111±13; *P*<0.001), in the part B from 75±5 to the maximum at 20 min (138±22; *P*<0.001), in the part C from 75±7 to the maximum at 15 min (123±24 beats per min; *P*<0.001). A dose-dependent change of gastric myoelectric activity was found after atropine administration. Two-peak increase of dominant frequency of slow waves was longer and more prominent in the part B (compared with higher and lower doses of atropine). All changes identified in dominant frequency were only minor and mostly fluctuated within the normal range.

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**P-32**

Remodeling of the myocardium in anthracycline cardiotoxicity: comparison of left and right ventricle

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Chronic anthracycline (ANT) cardiotoxicity represents a feared complication of cancer chemotherapy resulting in dilated cardiomyopathy and heart failure. The aim was to study molecular changes associated with myocardial remodeling of the left and right ventricle in response to chronic ANT treatment and post-treatment follow-up. Chronic cardiotoxicity was induced in rabbits with daunorubicin (DAU 3 mg/kg, weekly, 10 weeks). A week after the last drug dose, the animals were randomized to sacrifice or follow up for next 10 weeks. The chronic treatment with DAU led to significant LV systolic dysfunction and increase of plasma concentrations of cardiac troponin T. The degenerative changes were located mainly in the LV myocardium, while RV myocardium was significantly less affected. Gene expression of thin myofilament components revealed only moderate and rather late changes in the LV, whereas the RV was unaffected. In contrast, gene expression of thick myofilament components was markedly decreased in the LV. We also found significant and persistent titin down-regulation in the LV, while this was not the case of the RV. Interestingly, whereas important transcriptional factor GATA-4 was significantly down-regulated in the LV due to the treatment, CARP was inversely regulated. We also found significant and persistent up-regulation of desmin expression in the LV at mRNA level and even higher change at the protein level. Furthermore, molecular and morphological remodeling in the LV concerned also extracellular matrix with marked expression of collagens I and IV, whereas RV showed rather minor changes only. In conclusion, chronic ANT cardiotoxicity is associated with profound molecular remodeling of cardiomyocytes and extracellular matrix particularly in the LV, while changes in the RV are apparently less pronounced.

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**P-33**

Interactions of a set of antiviral drugs with organic anion transporter 1 and organic cation transporter 2

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Transporters play an important role in the fate of xenobiotics and endogenous chemicals in the body. Renal tubular secretion via organic anion transporter 1 (OAT1) and organic cation transporter 2 (OCT2) leads to remove metabolic waste, toxins and drugs in the urine. Interestingly, certain drugs such as antivirals can be eliminated via both transporters simultaneously. The goal of the study was to compare the interactions of a set of antiviral drugs with hOAT1 and hOCT2. The HeLa cell line transiently transfected with hOAT1 and the MDCKII cell line transiently transfected with hOCT2 were used for the experiments. The rate of inhibition of intracellular accumulation of tritium labelled typical substrate for OAT1 (para-aminophenupuric acid, 3′H-PAH) and for OCT2 (1-methyl-4-phenylpyridinium, 3′H-MPP+) induced by addition of two different concentrations of each tested antiviral drug was evaluated. Among the tested substances, the highest potency to inhibit hOAT1 exhibited tenofovir. Tenofovir and its ester prodrug tenofovir disoproxil fumarate (TDF) at concentration 100 μM inhibited accumulation of 3′H-PAH by 72% and 25%, respectively. Efavirenz, abacavir and carbovir inhibited the accumulation of 3′H-PAH approximately by 50% at concentration...
1000 μM. In hOCT2 cell model, efavirenz, abacavir and TDF at concentration 100 μM inhibited 3H-MPP+ accumulation approximately by 25%. Efavirenz and abacavir at concentration 1000 μM inhibited 3H-MPP+ accumulation approximately by 60%. In conclusion, the results demonstrated more frequent interactions of the tested antiviral drugs with hOAT1. In most cases, a considerable inhibitory effect was found in a relatively high concentration. Efavirenz, abacavir and TDF interacted with both tested transporters.

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P-34
Changes in ventilation and inflammatory parameters due to combination therapy at experimental meconium aspiration syndrome

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Meconium aspiration syndrome (MAS) with hypoxic respiratory failure is a serious neonatal disease. In this situation, exogenous surfactant administration is the treatment of the first choice. However, aspirated meconium may inactivate surfactant and reduce effect of the therapy due to initiation of local inflammation followed by lung edema, oxidation damage and increased expression of inflammatory mediators. To enhance efficacy of exogenous surfactant, an anti-inflammatory agent - glucocorticoid budesonide – was added.

New Zealand white rabbits with meconium-induced respiratory failure were divided into four groups (n=6 in each): without therapy (Mec), with surfactant (Surf), with budesonide (Bud), and with combined therapy (Surf+Bud). Respiratory parameters and indexes of oxygenation were registered for additional 5 hours. After sacrificing animals, lung edema (expressed as wet/dry weight ratio), and oxidative damage (thiobarbituric acid-reactive substances, TBARS) and levels of interleukins (IL)-1β, -6, -8 and TNFα in plasma were determined.

Combined Surf+Bud therapy rapidly improved oxygenation compared to other groups (P<0.05) and this effect persisted till the end of experiment. Combined therapy also reduced lung edema and TBARS (P<0.05). All used therapies decreased levels of IL-1β and IL-8 in plasma (P<0.05), but Surf+Bud had superior effect (IL-1β P<0.009, IL-8 P<0.003).

In experimental model of MAS, budesonide combined with exogenous surfactant improved respiratory parameters, lung edema, oxidative damage and inflammation. Thus, addition of anti-inflammatory agent may prevent inactivation of surfactant and provide more effective therapy of MAS.

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P-35
Differential gene expression of important factors in human epicardial adipose tissue and left ventricular myocardium in end-stage heart failure

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Aims. Epicardial adipose tissue is proposed to play a crucial role in coronary artery disease and atrial fibrillation. In this work we characterize gene expression of several important factors in epicardial adipose tissue (EAT) and left ventricular myocardium (LV) during development of heart failure (HF).

Methods. Samples of EAT and LV were collected from explanted hearts of 29 patients (average age 48±9, 25 men, 4 women). All patients had been diagnosed with end-stage HF and underwent heart transplantation. Causes of heart failure were as follows: dilated cardiomyopathy (15), coronary artery disease (10) and other causes (4). Samples were collected immediately after heart explantation and flash frozen in liquid nitrogen. qRT-PCR was used for gene expression analysis.

Results. Expression of calcineurin, nuclear factor of activated T cells 3 and platelet-derived growth factor B was significantly higher (170-196%, P<0.05) in EAT compared to LV. Expression of transforming growth factor β1 (TGFβ1), insuline-like growth factor 1 (IGF1) and endothelin 1 (ET-1) were several folds higher in EAT (230%, 1170% and 950% respectively, P<0.05) compared to LV. A significant 30% (P<0.05) decrease in expression of vascular endothelial growth factor A was observed in EAT. Several genes (IGF1, TGFβ1, ET-1) displayed the same differential gene expression profile in EAT and LV regardless of HF origin and/or diabetes mellitus diagnosis.

Conclusion. In our work, we identified a significantly different expression of several important factors in EAT
and LV of end-stage HF. Finding that EAT highly expresses a number of genes, it is likely that it plays an important role in heart disease, notably in heart failure.

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Abnormal respiratory function is not associated with altered gene expression of HGF and c-Met in monocrotaline induced pulmonary hypertension in wistar rats

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Background. An established animal model of pulmonary arterial hypertension (PAH) is the monocrotaline-induced pulmonary hypertension in rats. Respiratory function of monocrotaline treated rats has not been well documented. Additionally, hepatocyte growth factor (HGF) with its receptor c-Met plays a role in tissue regeneration. We hypothesized that experimentally induced PAH affects respiratory system in rats and this might be associated with alterations in HGF/c-Met signalling.

Methods. Group of 13 male Wistar rats was injected with monocrotaline (MCR; 60 mg/kg) and 7 control rats (CON) received vehicle. Animals were weighted routinely and vital functions were measured using MouseOx meter. After 4 weeks, rats were sacrificed when showing signs of dyspnoe, lethargy and marked weight reduction.

Results. MCR-treated rats showed a decrease in body weight when compared to controls (281±16 g vs. 325±34 g, P<0.01). Development of pulmonary hypertension was associated with significant increase of right ventricular weight to body weight ratio (MCR: 0.86±0.14 mg/g vs. CON: 0.50±0.03 mg/g, P<0.05) and a trend of increased lung to body weight ratio (MCR: 8.14±0.91 vs. CON: 6.29±0.68; NS). Oxygen saturation was significantly decreased in MCR group (91±2 % vs. 95±0.7 %, P<0.05) and a trend of increased ventilation rate in MCR group was present (113±6 brpm vs 100±2 brpm; NS). We found unchanged mRNA expression of HGF and a lack of mRNA expression c-Met in lung tissue.

Conclusion. Monocrotaline disrupts the cardiovascular and respiratory system of Wistar rats, which leads to the development of PAH. This is not associated with alterations in gene expressions of HGF and c-Met.

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Anti-inflammatory effects of budesonide in experimental model of acute lung injury

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Acute lung injury (ALI) and its more severe form - acute respiratory distress syndrome (ARDS) - are characterized by dysfunction of pulmonary surfactant, lung edema and inflammation. In the treatment of ALI/ARDS, lung-protecting ventilatory regimes and various anti-inflammatory agents may be used. To evaluate its possible benefits, intratracheal glucocorticoid budesonide was given into the lungs of experimental animals with ALI/ARDS.

Lungs of New Zealand white rabbits were repetitively lavaged with saline (30 mL/kg) to induce ALI/ARDS. Then, animals were treated with budesonide (Pulmicort, 0.25 mg/kg) or were left without therapy (n=6 in each group). All these animals were oxygen-ventilated for additional 5 hours. One group of non-ventilated animals served as healthy controls (n=6). After sacrificing animals, total and differential leukocytes in blood, total and differential cells in bronchoalveolar lavage fluid (BAL), lung edema (expressed as wet/dry weight ratio), and levels of interleukins (IL)-1β, -6, -8 and TNFα in plasma were determined.

Repetitive lung lavage increased total number of cells (P<0.05), particularly neutrophils and eosinophils (both P<0.001 in the BAL fluid, lung edema formation (P<0.001) and concentrations of pro-inflammatory cytokines (P<0.05) compared to healthy controls. Budesonide decreased number of neutrophils in BAL fluid (P<0.001), reduced lung edema (P<0.01) and showed trend to decrease pro-inflammatory cytokines in plasma (P>0.05).

Budesonide alleviated lung edema and inflammation in experimental model of ALI/ARDS, showing further perspectives of administration of this drug in the treatment of ALI/ARDS in patients.

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**P-38**

**Inhibition of PDE5 by tadalafil influences in vivo and in vitro airway reactivity in ovalbumin-sensitized guinea pigs**

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**Introduction.** Non-selective inhibition of phosphodiesterases (PDE) via methylxanthine derivatives (theophylline) has been traditionally used in the therapy of chronic inflammatory diseases associated with airway obstruction (e.g. bronchial asthma and chronic obstructive pulmonary disease - COPD). PDE5 inhibition is widely used in the therapy of erectile dysfunction, pulmonary hypertension as well as other cardiovascular diseases. However, the expression of PDE5 was confirmed in several immune cells, suggesting its potential role in allergic inflammation. The aim of this study was to evaluate the effect of one-week administration of selective PDE5 inhibitor tadalafil in experimentally induced allergic inflammation (model of allergic asthma) in guinea pigs.

**Material and Methods.** 24 male adult guinea pigs, divided into 4 groups, have been used in the study. Control group has been left without sensitization. The latter 2 groups have been sensitized with ovalbumin over two weeks and thereafter treated intraperitoneally for 7 days with tadalafil at the daily dose of 1.0 mg/kg b.w. or with vehiculum, respectively. Specific airway resistance measured in whole-body double-chamber plethysmograph has been used as a marker of in vivo airway reactivity. The in vitro reactivity of tracheal and lung smooth muscle has been tested using organ bath method.

**Results.** Sensitization with ovalbumin has led to significant increase in in vivo and in vitro airway reactivity. Tadalafil reduced both specific airway resistance after nebulisation of histamine, and in vitro airway reactivity to cumulative doses of acetylcholine in tracheal and lung tissue strips. These changes have been associated with suppression of haematological markers of inflammation and apoptosis in animals treated with tadalafil.

**Conclusions.** Selective PDE5 inhibition seems to play a significant role in allergic airway inflammation. However, its anti-allergic and possible anti-asthma potential needs further testing.

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**P-39**

**Transplacental transport of tenofovir and tenofovir disoproxil fumarate**

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Tenofovir disoproxil fumarate (TDF) is a prodrug form of tenofovir (TFV), a nucleotide reverse transcriptase inhibitor. TDF-containing regimens are recommended as the first line therapy in HIV infected patients, including women of childbearing age, and represent an acceptable option for prevention of mother-to-child transmission of HIV (PMTCT). TFV is classified among category B drugs; it is, therefore, important to have detailed knowledge on transplacental transport of TDF and TFV to assess safety and effectiveness of TDF treatment in pregnancy. P-glycoprotein (MDR1), breast cancer resistance protein (BCRP) and multidrug resistance protein 2 (MRP2) are the most important drug efflux ABC transporters that may affect transplacental pharmacokinetics by active transport of their substrates from fetal to maternal circulation. To avoid potential drug-drug interactions resulting in toxicity or failure of PMTCT it is necessary to describe TDF/TFV interactions with these transporters. The aim of study was to investigate transplacental transport of TFV and TDF employing the method of dually perfused rat term placenta. Using open-circuit perfusion system, transport of TFV or TDF was studied in both fetal-to-maternal and maternal-to-fetal directions. In case of TFV, we observed weak transplacental passage without involvement of capacity-limited mechanism. Conversely, the transplacental clearances of TDF were concentration-dependent in both directions. Employing closed-circuit perfusion system, we confirmed saturable transport of TDF. Moreover, application of common MDR1 and BCRP inhibitor, GF120908, resulted in significant suppression of TDF transport from fetus to mother, whereas application of MRPs' inhibitor, indomethacin, had no effect on TDF transport. In conclusions, both drugs seem to have restricted placental permeation from mother to fetus. Our findings suggest an important role of MDR1 and BCRP in transplacental transport of TDF. These interactions may contribute to potential drug-drug interactions in transplacental pharmacokinetic of this agent.

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P-40

The effect of chelator on receptor binding and stability in $^{177}$Lu-labeled anti-EGFR monoclonal antibodies in vitro

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Aim. Nowadays, monoclonal antibodies are used in therapy of various diseases. Due to very specific accumulation in the target tissue the antibodies could be utilized as carriers of radioisotopes to the tumors in case of targeted radioimmunotherapy and/or diagnosis. Nevertheless, essential properties of antibodies such as binding to the target or stability in the organism may be influenced by various structural parameters. In this study we have focused on potential effect of selected chelators on radiochemical quality and in vitro receptor binding capacity of two modified monoclonal antibodies.

Materials and Methods. We have selected two monoclonal antibodies - cetuximab and panitumumab, both ligands of epidermal growth factor receptor (EGFR). These antibodies were linked with three macrocyclic bifunctional chelators (DOTA, NOTA and PCTA) and radiolabeled with lutetium-177. The radiochemical stability of the preparations was checked in HPLC system and the binding rate to EGFR expressing cell lines (A431, HaCaT and HepG2) was examined.

Results. The method employed led to very stable radiolabeled preparations for each of selected chelators. Binding rate of labeled antibodies varied from 15% to 45% of applied dose according to used cell line. The results showed that the binding to the target cells was not influenced by the molecule of chelator.

Conclusion. We conclude that relatively recently developed chelators (NOTA and PCTA) could be useful for radiolabeling of anti-EGFR antibodies with lutetium-177 as well as well-established chelator DOTA. Thus NOTA and PCTA could be convenient tools in future preclinical studies with these radiopharmaceuticals.

P-41

Acute poisonings by tricyclic antidepressants reported to the toxicological information centre in Bratislava

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According to the National Toxicological Information Centre (NTIC) in Bratislava poisonings by antidepressants have an increasing tendency. NTIC has still quite often been consulted for advice on tricyclic antidepressants (TCA) exposures, in spite of the fact that they are not the first line therapy in the management of depression since the late 1980s. TCA are still valuable in the treatment of severe depression and are also used in the treatment of chronic pain syndromes, obsessive compulsive disorder and panic disorder.

Methods. TCA intoxications were analyzed on the basis of data gathered from telephone consultations and medical reports forwarded to the NTIC from the whole area of Slovakia in the years 2008-2012. The following data were analyzed: age, sex, intent of exposure (accidental or suicidal), the clinical severity, symptoms, substances co-ingested and treatment of intoxications.

Results. During the five-year period 940 cases of antidepressant intoxication were reported, including 126 TCA intoxications, mainly by dosulepin, amitriptyline and clozapramine. Intoxications in women (59%) were more frequent than in men. The highest number of poisonings was in the age range 30-39 years (22%). Intoxications with suicidal intent (89%) predominated. The most common poisoning severity score was PSS1 i.e. mild symptoms (38%). One lethal combined intoxication was recorded. From the total number of TCA poisonings 25 were caused by one drug and 101 by combination with other substances. The most commonly abused substances in combined poisonings were benzodiazepines, other antidepressants, antipsychotics and alcohol (21%). The most frequent symptoms of mono-intoxications by TCA were somnolence (32%), unconsciousness (20%) and tachycardia (20%). Treatment of TCA mono-intoxications consisted mainly of administration of activated charcoal (48%), gastric lavage (24%) and laxatives (20%).
Possibilities and risks of using phosphodiesterase inhibitors in treatment of meconium aspiration syndrome

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Ventilation support, administration of exogenous surfactant and inhalation of nitric oxide are standardly used for treatment meconium-induced acute lung injury in newborns. Additionally, there is a possibility to administer anti-inflammatory drugs such as phosphodiesterase (PDE) inhibitors. Aim of this study was to observe and to compare effects of non-selective PDE inhibitor aminophylline and selective PDE3 inhibitor olprinone on cardiovascular parameters.

Oxygen-ventilated and anesthetized animals received intratracheally 4 ml/kg of meconium suspension (25 mg/mL) or saline. Meconium-instilled animals were treated by intravenous administration of aminophylline (2.0 mg/kg) delivered at two doses at 0.5 and 2.5 h after meconium instillation, or olprinone (0.2 mg/kg) given at single dose at 0.5 h after meconium instillation, or were left without treatment as controls.

Changes of cardiovascular parameters (blood pressure, heart rate, heart rate variability) were evaluated within 5 min of administration, 5 min after finishing administration and within 5 hours after treatment. Markers of cardiovascular injury (aldosterone, gamma-glutamyltransferase (GGT), aspartate aminotransferase (AST), alanine aminotransferase (ALT)) and oxidation markers (TBARS, total antioxidant status) were determined in plasma.

Meconium instillation induced oxidative stress and elevated aldosterone and slightly increased GGT and AST levels. Aminophylline and olprinone reduced oxidation stress, however, both PDE inhibitors caused short-term increase in blood pressure and heart rate, whereas heart rate variability remained increased till the end of experiment.

As both treatments exerted comparable short-term cardiovascular changes, their use in the newborns with MAS should be carefully monitored.

Effect of fenugreek seeds enriched diet on endothelial dysfunction in mild diabetes and its relation to nitric oxide and epoxyeicosatrienoic acids pathway

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Fenugreek seeds are reported to have strong antihyperglycaemic and antioxidant properties. Our aim was to investigate effect of the fenugreek seeds enriched diet on endothelial function and its 2 potent regulators - NO and epoxyeicosatrienoic acids (EETs) pathways in rat model of mild diabetes.

Male Wistar rats (total n=40) were randomized into 4 groups: control (C), diabetes (D), fenugreek (F), fenugreek + diabetes (FD). Mild diabetes was induced by streptozotocin administration in dose 25 mg/kg/d i.p. for 3 consecutive days. 8-weeks long treatment in the form of a diet enriched with fenugreek seeds (5%) began 2 weeks after. Vascular reactivity of aortic rings was tested by acetylcholine and sodium nitroprusside. We analyzed mRNA expressions of epoxygenases (Cyp2j4, Cyp2c23) generating vasodilatatory EETs and ω-hydroxylases (Cyp4a2, Cyp4a3) producing vasoconstrictory EETs in aortas using qRT-PCR and protein levels of endothelial NO-synthase (eNOS) by Western blotting.

Fenugreek restored blunted endothelium-dependent relaxation (acetylcholine pD2 \( P < 0.001 \)). Endothelium-independent relaxation was not affected. Concomitantly, aortas from diabetic animals exhibited significantly lower levels of eNOS protein (\( P < 0.001 \)) and fenugreek was able to attenuate this decrease (\( P < 0.05 \)). mRNA levels of epoxygenases and ω-hydroxylases were not influenced either by diabetes or fenugreek treatment.

Fenugreek enriched diet normalized vascular function together with eNOS protein levels in diabetic aortas, but did not alter mRNA expression of EETs producing enzymes. Thanks to these effects, fenugreek may be beneficial as adjuvant therapy of diabetes mellitus.
Failure of brain energy metabolism after cerebral hypoxia-ischaemia in immature rats: in vitro mitochondrial and in vivo NMR study

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Introduction. Perinatal asphyxia resulting from decreased placental blood flow and/or gas exchange may lead to perinatal hypoxic-ischaemic (HI) encephalopathy. Brain injury after HI insult evolves over time, and different mechanisms are critical during the individual phases. Pathogenetic mechanisms of perinatal HI encephalopathy are now just beginning to be understood. Mitochondrial function is a critical factor that determines the mode of neuronal death. Many studies have been focused on investigating alterations of mitochondrial respiration, specifically oxidative phosphorylation (OXPHOS), triggered by HI insult. Particularly, in main interest is role of mitochondria in apoptosis, failure of energy metabolism, calcium signalling alteration, and reactive oxygen species generation during hypoxia and reoxygenation. Nowadays, non-invasive, non-destructive and real-time-operating techniques, namely magnetic resonance imaging and spectroscopy, are increasingly utilised in neurological and neonatology departments to confirm or refuse cerebral HI injury.

Methods. Seven-day-old Wistar rat pups of either sex were subjected to the HI procedure according to Rice et al. (1981) with modifications. Brain samples were collected to assess OXPHOS by means of high-resolution respirometry and non-invasive magnetic resonance measurements.

Conclusion. Better understanding of fundamental mechanisms of functional, biochemical and structural changes after HI insult may help in searching new strategies for effective neuroprotection in prophylactic and/or therapeutic intervention.

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Comparative study of newly synthesized copper complexes on their anti-inflammatory and antidiabetic activities in rats

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Copper(II) chelates type of Schiff base with different ligands were objects of considerable attention in many fields of bioorganic chemistry. Significant antiradical, anti-inflammatory, antipyretic as well as antidiabetic activities have been described previously. This work is focused on the study of both anti-inflammatory and antidiabetic activities of five mononuclear diaquabis(carboxylato)copper(II) complexes with different aminoacids which were projected in effort to alleviate generally high toxicity of the cuprates. The anti-inflammatory activity of the compounds was measured plethysmometrically by reduction of rat hind paw edema which was induced by a subplantar injection of carrageenan. The cytoprotective activity of the studied compounds on pancreatic β-cells was evaluated using modified in vivo oxidative stress model of alloxan-induced diabetes mellitus in rats. All i.p. administered compounds in dose of 50 μmol.kg⁻¹ of body weight in different extent influenced both examined pathologically conditions. The average antiinflammatory activity of the compounds ranged from 24.3 to 60.2% of the control values. The ability to decrease glucose level in acute experimental conditions as result of their cytoprotective properties in range 32.2 to 55.1% have been also found. The highest effects in both evaluated parameters showed compound IP-5, i.e. diaquabis(DL-β-aminobutyrate-N,O)-cupper complex (Fig.1). The antiradical activity of the compounds is obviously responsive for their protective antiphlogistic and antidiabetic properties. However, further studies for understanding mechanism of the cytoprotective action of the original compounds are considered.

Fig. 1. Relative anti-inflammatory and antidiabetic activities of the evaluated compounds.
Involvement of microRNAs in isoproterenol-induced cardiac damage and potential role in diagnosis

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Introduction. Isoproterenol treated rats, a well known model of myocardial damage caused by excessive beta-adrenergic stimulation is linked with dysfunction of contractile apparatus of the heart, due to expression shift of cardiac myosin heavy chain isoforms. Dysregulation may be associated with corrupted biogenesis of small endogenous regulatory RNAs, known as microRNAs.

Methods. 18-20 weeks old male Wistar rats were treated for 8 days with isoproterenol (ISO; 5 mg/kg; i.p.). Using modified real-time PCR, we analyzed in samples from left ventricular free wall, skeletal muscle (m. soleus) and plasma the expression or level of muscle-specific microRNAs: miR-1, miR-133a, miR-499 and heart-specific miR-208a.

Results. After 8-day beta-adrenergic stimulation the animals developed a decrease of left ventricular expression of all evaluated microRNAs (miR-1, -133a, -208a, -499) of 60% or more. Conversely, expression of these microRNAs in skeletal muscle didn’t change in comparison with the control group. Similarly to skeletal muscle, plasma level of muscle-specific microRNAs (miR-1, miR-133a, miR-499) didn’t change after isoproterenol. But the plasma amount of heart-specific miR-208a underwent a massive increase from virtually no presence to the level of more than 140-fold higher after isoproterenol treatment.

Conclusion. Highly elevated plasma level of cardiac-specific miR-208a could be a novel marker of heart damage caused, at least in case of beta-adrenergic stimulation. It also highlights the significant decrease of left ventricular microRNA levels of all evaluated microRNAs, miR-208a included. We can imply, that muscle-specific microRNAs (miR-1, -133a, -499) are not suitable as diagnostic markers presumably because their primary source is the 100 to 150 times more massive skeletal muscle compared to the weight of myocardium.

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Efflux of lamivudine in mate1 expressing MDCK cells

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Multidrug and toxin extrusion proteins (MATEs) and human organic cation transporters (OCTs) represent an important determinant for the renal and hepatic excretion of mainly cationic drugs. While OCT1 and 2 mediate drug uptake from the blood to the hepatocytes and renal proximal tubular cells, MATE1 ensures the efflux into bile and urine. Lamivudine, the nucleoside reverse transcriptase inhibitor, is one of the most widely used antiretroviral drugs applied in the treatment of HIV-1 infection. Although it is well known that the major route of lamivudine elimination is the excretion of unchanged drug into the urine, the precise mechanisms involved have not been described yet. Here we studied interaction of lamivudine with MATE1 transporter using MDCK cells stably expressing human OCT1, OCT2 and MATE1 as well as double transfected MDCK-OCT1/MATE1 and MDCK-OCT2/MATE1 cells grown in monolayers on cell culture inserts.

We observed significantly lower accumulation of lamivudine in all cell lines expressing MATE1, while the basolateral to apical transport of lamivudine was greater in the MATE1 expressing cells when compared to the parent and OCT monotransfected cells. Addition of mitoxantrone in a concentration selective for MATE1 inhibition (2μM) lead to significantly increased cellular accumulation and reduced the apically directed transport of lamivudine in the cells expressing MATE1.

Our data show that MATE1 affects transcellular transport of lamivudine indicating that this antiretroviral drug is a MATE1 substrate. Based on our results, we hypothesize that MATE1 contributes to the excretion of lamivudine in kidney.

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Oral quercetin is not able to revert catecholamine cardiotoxicity

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Catecholamines represent essential endogenous substances involved in homeostasis of various organ systems, with particular importance in the cardiovascular system. A synthetic catecholamine isoprenaline (ISO), with non-selective β-agonistic activity, has been used in high doses for induction of a state similar to acute myocardial infarction in humans. Despite the complex mechanism of ISO cardiotoxicity, some natural compounds have been shown to protect myocardium in this model.

This study was aimed at the detailed elucidation of the impact of oral pretreatment by flavonole quercetin on acute cardiac ISO toxicity.

23 Wistar:Han male rats of approximate weight 375 g were divided into four groups. Gastric gavage was used for oral premedication by quercetin (10 mg.kg\(^{-1}\)) or solvent daily for 7 days, followed by s.c. administration of ISO (100 mg.kg\(^{-1}\)) or solvent. Haemodynamic, ECG and biochemical parameters were analysed, including examination of myocardial histology and blood vessels responsiveness.

Quercetin pretreatment was not able to prevent majority of pathophysiological consequences caused by ISO (e.g., stroke volume decrease, cardiac troponin T release, QRS-T junction elevation, histological impairment), except for left ventricular end-systolic pressure which was normalized by quercetin in ISO treated animals. Interestingly, quercetin decreased responsiveness of isolated aorta on vasoconstrictor stimulus in the control animals.

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Experimental study of potassium and calcium ion channels in human pregnant labouring myometrium

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Myometrium of uterus is characterized by the presence of various ion channels that activity can be modulated pharmacologically using the specific agonists or antagonists. The experimental study was focused on potassium K\textsubscript{ATP} and BK\textsubscript{Ca2+}, and calcium CRAC ion channels in human pregnant labouring myometrium.

Samples of myometrium were taken from term pregnant women in which the pregnancy had to be terminated by Caesarean section. Subsequently the samples were processed in myometrial strips and placed in organ bath with Krebs-Henseleit solution. Myometrial contraction activity was evoked by the application of oxytocin. The amplitude of myometrial contractions was assessed after administration of agonist and antagonist K\textsubscript{ATP}, potassium ion channels - pinacidil and glybenclamide; agonist and antagonist BK\textsubscript{Ca2+}, potassium ion channels - NS1619 and tetraethylammonium; and CRAC ion channels antagonist 3-fluoropyridine-4-carboxylic acid.

Pinacidil significantly decreased the contractile activity of myometrium and its effect was significantly antagonized by glybenclamide. In contrast, NS1619 and its antagonist tetraethylammonium did not affect significantly the contractile activity of myometrium. 3-fluoropyridine-4-carboxylic acid did not have significant effect on the amplitude of myometrial contractions, too.

The function of K\textsubscript{ATP} and BK\textsubscript{Ca2+}, potassium ion channels in human term pregnant myometrium in labour is probably different. Down-regulation of the BK\textsubscript{Ca2+} potassium ion channels plays probably more important role to increase uterine contractility than K\textsubscript{ATP} potassium ion channels. Low expression of calcium CRAC ion channels did not affect the contractile activity of myometrium in experimental conditions.

ESF - Zvýšenie možností kariérneho rastu vo výskume a vývoji v oblasti lekárskych vied, VEGA 1/0062/11, VEGA 1/0127/13.
Off-label use of medicinal products in children is common, as few drugs are licensed for use in paediatric population. Concerns rise mainly due to the lack of knowledge about safety and effectiveness of drugs used off-label. Several studies have documented the association between adverse drug reactions (ADRs) and off-label drug use in children. Serious ADRs from off-label prescribing of medicinal products appeared in following therapeutic groups: allergens, dermatologicals and sex hormones. No study investigating the extent and characteristics of off-label prescribing in the context of ADRs in the Slovak Republic among children has been conducted yet.

A retrospective, cross-sectional analysis of spontaneous ADRs reported to State Institute for Drug Control in the Slovak Republic during 2006-2011 was performed. We included reports concerning drugs prescribed for patients younger than 18 years. Each ADR was classified according to seriousness and type of reaction. Off-label drug use was defined as prescription outside the licensed age group specified in Summary of Product Characteristics. We excluded ADRs related to vaccines, intravenous replacement solutions, blood products, dietary supplements.

Out of 1690 ADRs for children reported in the national database, 175 were analysed according to our inclusion criteria. We identified 14% serious, 70% non-serious ADRs and one fatal case. Most commonly suspected therapeutic group as a cause of ADRs were anti-infectives for systemic use (67%) which were also associated with the largest number of serious adverse drug reactions. 6% of reported ADRs were associated with off-label use. Serious ADRs occurred in two cases when off-label drugs were given.

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Effect of social stress on the reactivity of the aorta of young female rats with different predisposition to hypertension

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Effect of social stress on the reactivity of the aorta of young female rats with different predisposition to hypertension

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Genetic hypertension and social stress are supposed to be associated with changes in bioavailability of NO, which can be manifested by alterations in vascular reactivity. The aim of the work was to study the influence of crowding stress on endothelial function of the rat aorta of young normotensive Wistar-Kyoto rats (WKY) and spontaneously hypertensive rats (SHR). Five-week-old females of SHR and WKY rats were exposed to crowding for 2 weeks. Crowding was induced by reduction of living space from 200 cm²/100 g of body weight (controls) to 70 cm²/100 g of body weight. Endothelium-dependent (acetylcholine-induced) relaxation and noradrenaline-induced contraction of aortic rings were studied in vitro under isometric conditions. Blood pressure (BP) was measured using tail-cuff plethysmography. In the aorta, the NO synthase (NOS) activity was measured by conversion of [3H]-L-arginine to [3H]-L-citruline and superoxide production by the chemiluminescence method. In control rats, SHRs had significantly higher BP and increased NO and superoxide production in the aorta compared to WKY rats. However, no changes in aortic function were observed. Crowding stress failed to affect BP and vascular function either in SHR or WKY, it however increased significantly aortic NO production in both phenotypes. Superoxide production was increased by stress only in the aorta of WKY. In summary, crowding stress induced changes in NOS activity and superoxide production in the aortas of young female WKY and SHR rats, which however were not sufficient for manifestation of endothelial dysfunction.

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Expression of SDF-1/CXCR4 axis and stem cells markers in anthracycline-induced heart failure in the rat

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Introduction. Anthracyclines are effective antitumor drugs, but their clinical use is limited by dose-dependent cardiotoxicity. Stromal cell-derived factor-1α (SDF-1α) and its receptor CXCR4 have a crucial role in the mobilization and homing of the stem cells to ischaemic myocardium, with beneficial effect to myocardial repair. SDF-1 can be cleaved and downregulated by dipeptidyl peptidase-4 (DPP4).

Aim. To determine the expression of the stem cells markers and genes related to SDF-1/CXCR4 axis that could participate in the regeneration of damaged heart after anthracycline administration.

Materials and Methods. Daunorubicin was administered to male Wistar rats (15mg/kg, i.v., DAU). Control animals received vehicle. After 8 weeks, animals were sacrificed. Expression of stem cell factor (SCF), SDF-1α, CXCR4, DPP4, growth factors and markers of cardiac progenitor (c-kit and MDR1), endothelial progenitors (CD34 and CD133) and mesenchymal (CD44 and CD105) stem cells at mRNA level was determined by qRT-PCR in samples of left ventricles.

Results. We found decreased expressions of CXCR4 and DPP4 in hearts of DAU group. SDF-1α mRNA levels were unchanged. DAU group had also decreased expression of vascular endothelial growth factor (VEGF) and markers of endothelial progenitors and mesenchymal stem cells. CD44 expression was not altered. We also observed a decreased expression of cardiac progenitor cells marker c-kit and its ligand SCF, whereas the expression of MDR1, another cardiac progenitor cells marker, was increased.

Conclusion. Daunorubicin did not change the expression of SDF-1α, but it decreased expression of SCF, another chemotactic factor. This could reduce migration of stem cells. The observed decreased expression of stem cell markers could reflect their defective migration and impaired and decreased regeneration ability of the injured heart. Decreased expression of VEGF in the failing hearts could indicate a defective angiogenesis.

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Modified cellulose materials in the acute wound healing

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Wound healing is a dynamic and highly integrated process that represents important ability of organism to repair tissue damage in order to restore the proper function. Wound care procedures, mainly dressing techniques, have a great impact on the outcome of wound healing to achieve fast and antiseptic wound closure and improve regeneration of the impaired tissue.

Cellulose is a natural polysaccharide that is used in medicine in many indications for its haemostatic and potential bacteriostatic properties. The effectiveness of newly synthesized modified cellulose derivatives based on carboxymethyl cellulose was examined, using a skin wound model in rats, with a focus on the rate of healing (wound diameter), tissue reaction and determination of the cytokines involved in the wound healing process.

A total of 60 Wistar laboratory rats were divided into 4 groups of 15 animals. Under total anesthesia, skin circle excision (1.5 cm in diameter) through all the cutaneous layers in the suprascapular region was performed. Carboxymethyl cellulose (HHT), carboxymethyl cellulose with iodine (HTB), and as the reference substance carboxymethyl cellulose with silver (AAG) were used; control group was left without primary dressing. The tested material was applied on the wound, thereafter fixed by stapler. Third of the animals were destroyed after 2, 7 and 14 days from the experiment start date. Before the euthanasia, blood was obtained by cardiac puncture for biochemical examination. Wound and its surroundings were macroscopically observed, skin samples were taken and subjected to pathohistological and molecular biological examination. Total destruction score as a degree of inflammation in epidermis, dermis and hypodermis was histologically determined. The cytokines TNF-α, TGF-β1 and VEGF were detected by the western blot.

Based on this pre-clinical in vivo experiment, HHT and HTB derivatives showed better characteristics in comparison to conventionally used AAG. They reduced inflammatory response in the skin with decreased level of proinflammatory cytokines, and support angiogenesis as well as the consequent regeneration of the impaired tissue.

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Teaching clinical pharmacology in baccalaureate nursing programs in the Czech Republic

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troduction. Clinical pharmacology is an important subject of study in university education of general nurses. Courses for nursing students should be more clinical than those for medical students and should provide information on problems encountered by nurses during drug administration. The aim of this study was to analyze and compare the pharmacology courses in baccalaureate nursing programs at Czech universities with emphasis on the specific needs of nursing practice.

Methods. Pharmacology training courses of all Czech universities providing graduate education for general nurses were analyzed. Both qualitative and quantitative methods of document analysis were used. The online curricula and course syllabuses were obtained from open sources. Moreover, assessment of didactic effectiveness of recommended pharmacology textbooks for graduate nursing courses was performed.

Results. Teaching Pharmacology in the graduate program of nursing takes place in a total of 14 universities in the Czech Republic. The most common length of the course was between 20 – 29 hours (in 42.86% of curricula). Large differences were also found in the content of the subject. Nearly hundred-percent representation reached the fundamental issues of pharmacology, such as general principles of pharmacokinetics (92.86%) and pharmacodynamics (78.57%) and special pharmacology selected chapters: treatment of bacterial infections (85.71%), drugs affecting cardiovascular system (92.86%), analgesics (78.57%) and drugs affecting autonomic nervous system (71.43%). In contrast, chapters from the clinical pharmacology were represented only in minute quantities. This was mainly pharmacotherapy of pain (28.57%) and pharmacotherapy in the elderly (21.43%), children (14.29%) and pregnant and lactating women (21.43%). The didactic effectiveness of the most frequently used Czech textbook, Pharmacology for Students of Health Sciences, reached 61.11%.

Conclusion. Significant differences between pharmacology courses for nurses were found in the length of courses, their position in the curriculum, course contents and forms of examination. Didactic effectiveness of used textbooks is insufficient in comparison with those of English-speaking countries. In particular, insufficient attention is paid to specific nursing problems of drug use.
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P-56
Antibacterial effect of silver nanoparticles in experimental skin infection

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Introduction. Antibacterial activity of silver nanoparticles is well known. At present, the investigation of this phenomenon is intensifying because of the increase of bacterial resistance to antibiotics, caused by their overuse. The aim of this study was to evaluate antibacterial activity of liquid and semisolid formulation of silver nanoparticles on the model of an experimental rat skin infection.

Methods. Male specific pathogen free Wistar rats were used and during experiments housed in individually ventilated boxes. Full-thickness skin wounds were made by round scalpel under general anesthesia. Wounds were inoculated with S. pyogenes strains, obtained from 24-hour culture inoculated to 20 ml of meat-pepton suspension and incubated for 24 h at 37 °C. Topical treatment was applied once daily for a further 6 days. Animals were divided into 3 groups (n=5), treated by Ag-nanoparticles solution, erythromycin solution or placebo or Ag-nanoparticles ointment, neomycin+bacitracin ointment and placebo, respectively. Macroscopic healing assessment and smears for bacterial counts were performed at days 2, 4 and 7 of the experiment. S. pyogenes strains were identified by Pastorex Strep set.

Results. At the day 7 of the experiments, silver nanoparticles in both liquid and ointment formulation significantly decreased bacterial counts in comparison with placebo. Differences in macroscopic wound healing between treated and placebo groups were not significant.

Conclusion. Silver nanoparticles both in solution and in ointment show antibacterial activity against experimental Streptococci skin wound infection. On the contrary, no effect on the velocity of wound healing was found.

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P-57
Effect of sulforaphane on enzyme activities of human liver microsomal cytochromes P450

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Sulforaphane (SFR) is a phytochemical, which belongs to the family of isothiocyanates and is formed from glucoraphanin (glucosinolate in broccoli) by the action of myrosinase. The largest amount of SFR occurs especially in broccoli, broccoli sprouts, but it has been also found in other cruciferous vegetables such as cabbage, cauliflower and kale. SFR, biologically active substance of broccoli, may protect against various types of cancer, diabetes or cardiovascular disease. SFR is a possible inhibitor of some major cytochrome P450 enzymes (CYP), which are taking part in a variety of important reactions of drug metabolism in humans. For this reason, the influence of SFR on activity or inhibition of some selected human hepatic microsomal CYPs (1A2, 2A6, 2B6, 2C9, 2C8, 2D6, 2E1 and 3A4) was studied. Our experiments confirmed recent data on inhibition CYP2E1 enzyme activity (chlorzoxazone 6-hydroxylation) by SFR. Significant decrease was also observed in activities of CYP3A4 (testosterone 6β-hydroxylation) and of CYP2D6 (bufuralol 1’-hydroxylation). Mechanisms of inhibition remain to be elucidated by further studies.

P-58
Novel analogues of dexrazoxane and ADR-925 for protection against anthracycline cardiotoxicity

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Cardiotoxicity as a serious late side-effect compromises the clinical usefulness of anthracyclines (ANT).
Dexrazoxane (DEX) is the only clinically established cardioprotective agent, but its use is very limited. Traditionally, iron-mediated oxidative stress is believed to be the main cause of AN1T cardiotoxicity, and DEX-induced cardioprotection is attributed to its hydrolysis product ADR-925 that can chelate free iron, and thus prevent cardiac oxidative injury. However, parent structure of DEX is also a catalytic inhibitor of topoisomerase II (TOP2). In this study, novel analogues of DEX (MK-15, ES-5) and ADR-925 (KH-TA4, JR-159) were synthesized, and their protective activities against daunorubicin (DAU) cardiotoxicity were examined both in vitro and in vivo. In contrast to DEX, none of the novel agents significantly protected against the DAU cardiotoxicity, and they also displayed no significant antiproliferative properties. On the other hand, KH-TA4 protected cardiomyocytes from model oxidative damage, whereas DEX was ineffective. The binding of iron or its mobilization from cardiomyocytes not differed from DEX, but unlike DEX the analogs lacked TOP2 inhibition properties. In conclusion, our data indicate that rather than by antioxidative properties, the interaction with TOP2 may be involved in anthracycline cardiotoxicity as well as it may be indispensable for effective pharmacological cardioprotection.

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P-59
Silybin affects the liver microsomal CYP2C6 in HHTG rats
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This study was aimed at investigate of the effect of silybin on expression and activity of rat CYP2C6. This cytochrome P450 2C6 is considered to be a counterpart of human CYP2C9, which metabolizes commonly prescribed drugs, such as ibuprofen, diclofenac or warfarin.

Male hereditary hypertriglyceridemic rats (accepted model of metabolic syndrome) were fed: 1) standard laboratory diet (STD), 2) high cholesterol diet (HCD = STD + 1% of cholesterol w/w + 10% of lard fat w/w), 3) high cholesterol diet with silybin (0.5% w/w) for 21 days. Expression of cytochrome P450 2C6 was measured in liver using real-time PCR (at mRNA level) and Western blotting (at protein level). Formation of diclofenac metabolites (typical marker substrate of CYP2C6 enzyme activity) was analyzed using HPLC with UV detection.

Silybin in hereditary hypertriglyceridemic rats on HCD diet significantly increased activity of CYP2C6 and its expression on mRNA level. Expression of CYP2C6 on protein level was affected by silybin consumption insignificantly. Our results suggested that CYP2C6 is up-regulated by silybin in hereditary hypertriglyceridemic rats on high cholesterol diet.

Because cytochrome P450 2C6 is considered to be a counterpart of human CYP2C9, the results obtained open the possibility that in humans silybin may affect the metabolism of drugs metabolized by this cytochrome P450. Further studies are needed to elucidate the effects of silybin on CYP2C9 in humans suffering from metabolic syndrome.

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P-60
Study of renal clathrin-independent endocytosis of radiolabeled receptor-specific bombesin analogues
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Bombesin (BN) is a neuropeptide with high affinity for the gastrin-releasing peptide (GRP) receptor, which is overexpressed by a variety of cancers and provides an attractive target for BN/GRP receptor scintigraphy and radionuclide therapy. However, renal accumulation of radiopeptides resulting in toxicological injury may limit potential clinical use of the tested compounds. Renal endocytosis of the bombesin analogues has usually been studied only as a receptor mediated process. But we tried to investigate the role of macropinocytosis, one of clathrin-independent endocytosis in the renal uptake.

As a fluid-phase endocytosis probe, FITC-labeled dextran (FD) was used. We measured the fluorescence of macropinocytosed FD at various concentrations using LLC-PK1 cells and then determined the influence of escalating concentrations of bombesin on 0.5 mg/mL FD macropinocytosis. 5 μM rotterlin was used as a selective inhibitor of macropinocytosis.
Bombesin increased the FD endocytosis in LLC-PK cells up to a concentration of 0.5 mg/ml. At a concentration of 1 mg/ml, the FD endocytosis was declined. Rottlerin which is selective, rapid-acting and irreversible inhibitor of fluid phase endocytosis, caused significant lower uptake of $^{177}$Lu labeled bombesin analogues compared to the cells treated with bombesines only. The inhibition rate was more than 80% for $^{177}$Lu-PCTA-[Lys]$^3$ bombesin, 45.8% for $^{177}$Lu-DOTA-[Pro$^1$,Tyr$^4$]bombesin and 38.5% for $^{177}$Lu-DOTA-[Lys]$^3$ bombesin.

Based on our results the vesicular uptake is associated with macropinocytosis of the bombesins, but its contribution differs according to the used chelating component or substitution in bombesin molecule. Bombesins are under this experimental conditions internalized into large macropinosomes. We can conclude, that fluid phase endocytosis, which is usually called as clathrin-independent endocytosis may play an important role in the accumulation of radiolabeled bombesin analogues in the renal tubular cells.

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P-61
Sedative and anxiolytic properties of flumazenil
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Flumazenil acts as a benzodiazepine receptor antagonist or partial agonist. It competitively inhibits the activity at the benzodiazepine recognition site on the GABA/benzodiazepine receptor complex, thereby reversing the effects of benzodiazepines.

Our experiments in rabbits showed that administration of flumazenil (0.1 mg/kg) is associated with sedative and anxiolytic effect, which was, in some parameters similar to that of midazolam (0.5 mg/kg). In those rabbit, the loss of righting reflex was achieved approximately after 3 minutes irrespective of the administration of midazolam of flumazenil. On the other hand, midazolam caused more profound decrease of blood pressure and pulse rate. These results were confirmed by ultrasonic vocalization test of anxiety in rats. The rats were administered with various doses of flumazenil (0.1, 1 and 10 mg/kg) and an anxiolytic effect of flumazenil was observed. The study was placebo controlled and three different doses of flumazenil based on literature data were used. While saline had no influence on the duration and length of recorded amplitudes, flumazenil significantly and dose-dependently decreased the total number of vocalizations.

There are plenty of preclinical or clinical data showing reversal of sedation by flumazenil induced by benzodiazepines. On the contrary, there are little data showing the effect of flumazenil or other antagonists itself. These studies seem to be in contrary with the effect of flumazenil in humans, where it is believed to possess mainly anxiogenic effect. It seems that in the individuals, who exhibit anxiogenic behavior or in individuals with anticipation anxiety, flumazenil acts as an anxiolytic agent, while in individuals without any signs of anxiety, flumazenil can really act as anxiogenic agent.

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P-62
Boldine enhances bile production in rats
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Boldine is the major alkaloid from Chilean boldo tree, and is used in traditional medicine to support bile production, but the scientific data for this effect are lacking. We therefore analyzed choleretic potential of the compound including its possible molecular background. The immediate and long-term effect of boldine was evaluated in rats either during 2h i.v. infusion or after 28-day oral pretreatment. Constant infusion of boldine instantly increased the bile flow 1.4-fold and maintained it stably higher throughout the administration. This effect was not associated with
the corresponding increase in bile acid or glutathione biliary excretion, which indicates that acute choleretic effect of boldine is not related to the stimulation of either bile acid dependent or independent mechanism of bile formation, and it points to osmotic activity of compound itself. Therefore, we analyzed also the concentration of boldine in bile and performed the bile clearance study after bolus dose administration of the agent. Indeed, we found a strong relationship between the elevation of bile production and the actual bile excretion of boldine. Nevertheless, after long term pretreatment, when the bile collection study was performed 24 h after the last administration of boldine, the production of bile was accelerated despite undetectable levels of the compound in bile or plasma, and without influence on biliary glutathione and bile acid excretion. In conclusion, our study demonstrated immediate choleretic activity of boldine, which depended on actual concentration of the agent in the bile. Mechanism of increased bile production during long-term administration of boldine requires further analysis.

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P-63

Activation of NO and cytokines by low-molecular weight fractions of lysates from G+ and G− bacteria

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Immunostimulatory effects of bacterial lysates and their fractions differing in the content of molecules of specified molecular weight (MW) were investigated. The lysates were prepared by passing the probiotic bacteria Lactobacillus casei DN-114001 and Escherichia coli strain Nissle 1917 through the French press followed by lyophilisation. The lysates fractions differing in the content of molecules of specified MW range were prepared using the MW cutoff microfiltration (centrifugal devices Amicon®, Millipore). Four classes of MW cutoff fractions (MWCF) were obtained. They contained molecules ≤ 100 kDa, ≤ 10 kDa, ≤ 3 kDa. The effects of original lysates and MWCF on the in vitro production of nitric oxide (NO) and cytokines interleukin-1β and tumour necrosis factor-α were analysed in rat peritoneal cells (2 x 10⁶/mL). Nitrite and cytokines levels were determined at the 24-h interval of culture, using Griess reagent and ELISA, respectively.

It was found that all MWCF possessed the NO- and cytokine-stimulatory activity, although less expressed than was the activity of original lysate. The MWCF of Gram-positive L. casei were more effective than MWCF of Gram-negative E. coli. The most of the NO-stimulatory potential was found to be attributable to the molecules of ≤ 3 kDa. The L. casei MWCF-3 kDa accounted for approximately 80% of the NO-stimulatory effect of MWCF-100 kDa.

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P-64

Immunosuppressive pyrimidines: structure-activity study

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We synthesized novel series of pyrimidine derivatives and analysed their immunobiological properties. Effects of compounds on immune-activated production of nitric oxide (NO), prostaglandin E₂ (PGE₂) and cytokines (interleukin-1, interleukin-6, tumour necrosis factor-α) were screened under in vitro conditions using mouse peritoneal cells. NO production was activated by lipopolysaccharide (LPS) plus interferon-γ (100 pg/mL/500 pg/mL, respectively), PGE₂ by LPS (10 ng/mL), and cytokines by LPS (5 μg/mL). Pyrimidine derivatives were applied as 50 μM aqueous solutions, concomitantly with the triggering stimuli. The effects were evaluated 24 h afterwards.

The compounds exhibited inhibitory mode of action which was strongly dependent on their structure. While 2-amino-4,6-dihydroxypyrimidines were ineffective as NO inhibitors, 2-amino-4,6-dichloropyrimidines suppressed NO production by 50%. The highest inhibitory effects were displayed by 4,6-dichloro-2-{(N,N-dimethylamino) methyleneamino}pyrimidines and 4,6-dichloro-2-formamidopyrimidines which inhibited production of NO by approximately 95%. The 5-position substituents remained without any significant impact on NO production. The NO-inhibitory IC₅₀s of most of them were ≤ 5 μM.

The compounds were devoid of cytotoxicity.
Besides NO, the pyrimidine derivatives also suppressed, though less effectively, production of PGE2 and cytokines. The mechanism of the action remains to be established.

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P-65

Quantification of drug-drug interactions between calcineurin inhibitors and antimicrobial agents in kidney transplant recipients in routine clinical practice

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Patients. We performed a retrospective analysis of TDM measurements in the past 5 years at a single transplant centre in the University Hospital in Olomouc, Czech Republic. A total of 4,024 measurements of trough concentrations (C0) of CIs and MPA were included in the analysis made in 111 male and 70 female kidney transplant recipients (181 in total).

Patients were aged 13 to 72 years (average 45.7), with the exception of two patients, all were recipients of grafts from deceased donors. Average time from transplant was 5.6 years (0.4 to 18).

Drug measurements were obtained from the hospital information system, information on prescription was ascertained from prescription database and patient clinical records (including dosing instructions and specific temporary instructions concerning co-prescription of interacting medication).

CI levels were expressed as normalised dose required to reach a unit concentration and a linear regression model was constructed to quantify effects of various interacting factors (e.g., age, sex, prednisone dose, MPA dose, body weight).

Results. A model explaining the variability of ND was constructed as follows:

\[
\text{Normalised dose of tacrolimus} = 7.09e^{-3} - 9.98e^{-4} \times \text{MPA} + 2.13e^{-4} \times \text{Tx} - 5.92e^{-4} \times \text{ATB} - 2.56e^{-3} \times \text{INTER} + 1.52e^{-4} \times \text{DOSE} - 2.53e^{-5} \times \text{AGE}
\]

Where MPA is the dose of mycophenolate mofetil in mg/kg body weight, Tx is time from transplantation in years, ATB is 1 if the patient is treated with an antimicrobial, INTER is 1 if the patient is treated with an interacting antimicrobial, DOSE is the dose of tacrolimus in μg/kg of body weight, and AGE is patient’s age in years.

Presence of an interacting antimicrobial prescription was a highly significant predictor of CI dose/level ratio and lead on average to a 36% reduction in the dose required to achieve therapeutic levels \( (P<0.01) \). In 60% of the cases the dose was reduced by the prescribing physician. Antimicrobials without a reported interaction (control group) led to a 8% reduction in the dose/level ratio \( (P<0.01) \).

Conclusions: In real-practice settings we quantified the effects of antimicrobial prescription on CI levels and maintenance of therapeutic levels. We constructed a predictive model that describes some of the variability in CI levels as seen in routine monitoring after kidney transplant.

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P-66

Influence of cranberry and polyphenon E on gene expression of cytochromes P450 (CYP) in mouse liver

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Polyphenon E is a green tea catechin extract. Many of the effects of green tea are thought to be due to catechin, an especially epigallocatechin gallate (EGCG) as the main component accounting for 50% of the material. American cranberries (Vaccinium macrocarpon) are...
a particularly rich source of phenolic phytochemicals, including phenolic acids and flavonoids. Polyphenon E and cranberry have influence on human health and may interfere with the way the body processes certain drugs using the liver’s cytochrome P450 enzyme system.

Eight months old male NMRI mice were used to this experiment and were divided into three groups: The controls (A) and to two an experimental group (B, C). Group B was treated with 2% (w/w) cranberry extract mixed with diet for 4 weeks. Group C were fed by diet enriched by Polyphenon E in concentration 0.1% (w/w) for 4 weeks. Control group (A) was fed by standard diet. Effect of both compounds on cytochromes P450 expression was studied using real-time RT-PCR. Relative expression (RE) of a gene of interest was calculated by ddCT method against a selected reference gene (Hprt and 18S rRNA). Specific primers of mice CYPs enzymes were selected with high sequence similarity (orthologous) to human CYPs enzyme.

Our data showed induction of cytochromes P450 expression in the group B which were fed by diet enriched by cranberry extract in concentration 2% (w/w) for 4 weeks. Regulation of cytochromes P450 expression and activity is a multi-level process that is currently not completely understood.

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Activation of NO and cytokines by low-molecular weight fractions of lysates from G$^+$ and G$^-$ bacteria

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Background. Probiotic bacteria are recognized for favourable effects in the treatment of various pathologies, mainly of gastrointestinal tract, such as ulcerative colitis, pouchitis and diarrhea. The clinical effects have been suggested to result at least in part from immunobiological activities of both live and dead bacteria. The aim of present experiments was to investigate whether and to what extent the spectrum of immunobiological properties of probiotics might be contributed to by molecules characterized by the low-molecular weight. Immunostimulatory effects of bacterial lysates and their fractions differing in the content of molecules of different size were investigated.

Methods. The lysates were prepared by passing the probiotic bacteria Lactobacillus casei DN-114001 and Escherichia coli strain Nissle 1917 through the French press followed by lyophilisation. The lysate fractions differing in the content of molecules of specified molecular weight (MW) range were prepared using the MW cutoff microfiltration (centrifugal devices Amicon®, Millipore). Four classes of MW cutoff fractions (MWCF), devoid of polymeric components of bacterial cell walls such as lipopolysaccharide, lipoteichoic acid and peptidoglycan were obtained. They contained molecules ≤ 100 kDa, ≤ 50 kDa, ≤ 10 kDa, or ≤ 3 kDa. The effects of original lysates and MWCF on the in vitro production of nitric oxide (NO) and cytokines interleukin-1β (IL-1β) and tumour necrosis factor-α (TNF-α) were analysed in rat peritoneal cells (2 x 10⁶/ml). Nitrite and cytokines levels were determined at the 24-h interval of culture, using Griess reagent and ELISA, respectively.

Results. It was found that all MWCF possessed the NO- and cytokine-stimulatory activity, although less expressed than was the activity of original lystate. The MWCF of Gram-positive L. casei were more effective than MWCF of Gram-negative E. coli. The most of the NO-stimulatory potential was found to be attributable to the molecules of ≤ 3 kDa. The L. casei MWCF-3 kDa accounted for approximately 80% of the NO-stimulatory effect of MWCF-100 kDa. Irrespective of the bacterial origin, about 42% of the IL-1β-enhancing activity of the crude lystate was contained in the MWCF-3 kDa. The MWCF-3 kDa was responsible for 22% and 52% TNF-α-stimulatory effects of the crude lysates obtained from L. casei and E. coli crude lysates, respectively.

Conclusions. The present results provide an unequivocal evidence showing that bacterial lysates contain chemical entities with MW < 100 kDa possessing immunostimulatory properties. They are present in both G$^+$ and G$^-$ bacteria. The molecules with MW ≤ 3 kDa are obviously the major contributors to the overall effect. Their share can be estimated to be approximately 55-85%, in dependence on the response and the type of bacteria. The findings warrant further studies which should identify chemical species and determine quantitative aspects of their occurrence in samples of different bacterial origin.

Key words: probiotics, lysate, microfiltration, nitric oxide, cytokines

INTRODUCTION

The major medical indications of probiotic bacteria are pathologies of gastrointestinal tract, such as ulcerative colitis, pouchitis and diarrhea. The favourable clinical effects have been suggested to result at least in part from a plethora of their immunobiological activities. Probiotics have been shown to enhance mainly the secretion of Th1 cytokines and regulatory cytokine interleukin-10 (IL-10) (ref.23). Both Gram-negative (G$^-$) and Gram-positive (G$^+$) probiotics are also prominent activators of nitric oxide (NO) biosynthesis4,5. The ability of probiotics to modulate the innate and acquired immune responses is presumed to be due to the complex macromolecules represented by immunobiologically highly active components of their outer membrane such as lipoteichoic acid (LTA) of G$^-$ bacteria, lipopolysaccharide (LPS) of G$^+$ bacteria, and peptidoglycan (PGN) occurring mainly in G$^+$ species. The aim of present experiments was to investigate whether and to what extent the spectrum of immunobiological properties of probiotics might be contributed to by molecules characterized by the low-molecular weight. For this purpose, the microfiltration fractions of lysates from Lactobacillus casei DN-114001 and Escherichia coli strain Nissle 1917 varying in the content of chemical entities of different molecular weight were prepared. Their ability to enhance production of NO and cytokines interleukin-1β (IL-1β) and tumour necrosis factor-α (TNF-α) was analysed.
Fig. 1. *In vitro* production of NO by rat peritoneal cells (2 x 10⁶/mL) stimulated with lysates and their molecular weight cutoff fractions (MWCF) obtained from the probiotic strains of *Lactobacillus casei* (A) and *Escherichia coli* (B). Concentration of nitrites was evaluated at the interval of 24 h of culture using Griess reagent. The points are means ± SEM. Each figure represents one of two identical experiments.

Fig. 2. Secretion of cytokines interleukin-1β (IL-1β) (A) and tumour necrosis factor-α (TNF-α) (B) by rat peritoneal cells (2 x 10⁶/mL) stimulated *in vitro* with lysates and their molecular weight cutoff fractions (MWCF) obtained from the probiotic strains of *Lactobacillus casei* and *Escherichia coli*. Concentration of cytokines was assayed at the interval of 24 h of culture using ELISA. The points are means ± SEM. Each figure represents one of two identical experiments.

MATERIAL AND METHODS

**Bacteria, lysates, and low-molecular weight lysate fractions**

*Lactobacillus casei* DN-114001 (*L. casei*) was obtained from the Danone Institute, France. *Escherichia coli* Nissle 1917 (*E. coli*; Mutaflor, DSM 6601, serotype 06:K5:H1) was supplied by Dr. J. Schulze (Ardeypharm GmbH, Herdecke, Germany).

The crude lysates were prepared from bacteria resuspended in distilled water. They were disrupted by passing three times through a French press (1500 psi), and then lyophilized and diluted to a working concentration of 30 mg/mL. To kill possible remnants of viable bacteria, the lysate was heated to 60 °C for 30 min. The sterility of preparations was checked by both aerobic and anaerobic cultivation. Ultimately, the lysates were centrifuged and passed through the 0.22 μm syringe filters.

The fractions of this lysate were further treated using the Amicon® Ultra 0.5 mL centrifugal microfiltration devices (Millipore Corp., Billerica, MA). This procedure allowed to prepare four classes of molecular weight cutoff microfiltrates (MWCM) containing chemical entities characterized by MW ≤ 100 kDa, ≤ 50 kDa, ≤ 10 kDa, and ≤ 3 kDa. It was shown previously that even the 100 kDa microfiltration eliminated the biologically active bacterial polymers such as LPS, LTA and PGN (ref.6).

**Animals and cells**

Peritoneal cells were obtained by lavage of female Wistar rats (8-10 wks old; AnLab, Prague, CZ) with 16 mL of PBS. The cells were resuspended in complete culture medium, and seeded into 96-well round-bottom microwells in 100μL volumes, 2 x 10⁵ cells/well. Cultures were maintained at 37 °C, 5% CO₂ in humidified Heraeus
incubator. Complete RPMI-1640 culture medium (Sigma-Aldrich) contained 10% heat-inactivated foetal bovine serum, 2 mM L-glutamine, 50 μg/mL gentamicin, and 5 x 10⁻⁵ M 2-mercaptoethanol (all Sigma-Aldrich). Each experimental variant was run in duplicate.

The animal welfare and all experimental procedures have been approved by the Institution Animal Ethics Committee.

NO assay
The cells were cultured 24 h in the presence of bacterial preparations. The concentration of nitrites in cell supernatants was taken as a measure of NO production. It was detected by the Griess reagent. A nitrite calibration curve was used to convert absorbance to μM nitrite.

Cytokine assay
Concentration of cytokines in supernatants was determined at the interval of 24 h of culture using the ELISA kits, and following the manufacturer instructions (R&D Systems, MN).

Statistical analysis
Analysis of variance with subsequent Bonferroni multiple comparison test, and graphical presentation of data were done using the Prism program (GraphPad Software, CA). The dose-response relationship was evaluated by means of area under curve (AUC).

RESULTS

NO production
Spontaneous production of NO by rat peritoneal cells was approximately 10 μM. It was substantially and dose-dependently enhanced by both original lysates and the lysate-derived molecular weight cutoff microfiltration fractions (MWCF) prepared from the probiotic bacteria *L. casei* and *E. coli*. The original, *i.e.* non-microfiltered lysates were more effective than those from *L. casei* (Figs 1A and 1B, respectively).

The most potent activators of NO production proved to be the MWCF of *L. casei* origin (Fig. 1A). Evaluated by means of AUC, the effects of the MWCF-100 kDa and MWCF-3 kDa were less expressed than those of the original lysate (Table 1).

The similar NO-augmenting tendency was exhibited by MWCF derived from the *E. coli* lysate (Fig. 1B). Nevertheless, in contrast to the very high efficacy of the original *E. coli* lysate, the NO-stimulatory effects of
MWCF were weak, much weaker than those of *L. casei*. The onset of the NO-enhancement after the *L. casei* MWCF became apparent when they were prepared from 5-10 μg/mL of the original lysate and NO production increased substantially towards higher MWCF concentrations. At least 10-fold higher amount of the original lysate was required to obtain the *E. coli* MWCF that were capable to augment the NO production (Fig. 1A versus 1B). Only the effect of MWCF-100 kDa was significant (*P*<0.05) if MWCF were prepared from the 50 μg/mL of lysate. When prepared from the 100 μg/mL of lysate, all MWCF-100, -50, -10, and -3 kDa did induce significant production of NO (*P*<0.001, <0.01, <0.05, and <0.05, respectively). Yet, the response remained very low (Table 1).

**Cytokine secretion**

The original lysates prepared from both *L. casei* and *E. coli* probiotics activated the secretion of cytokines (Fig. 2). The original lysates of *L. casei* were significantly more effective than those of *E. coli* in activating both IL-1β (*F*/*k* = 10.84, *P* = 0.011) and TNF-α (*F*/*k* = 64.53, *P* <0.0001) production.

The secretion of cytokines was statistically significantly enhanced by MWCF as well (Fig. 2), though less effectively than by the original lysates (Table 1).

**DISCUSSION AND CONCLUSIONS**

At present, reliable data on the diversity and chemical nature of small molecules produced by different types of bacteria and constituting the lysates are largely missing. Based on the available evidence, one of plausible candidates for the immunostimulatory effects of lysate MWCF might be the 492 Da molecule of MDP that has been shown to be the minimum constituent responsible for many biological activities of PGN (ref.8-12). However, the participation of MDP on immunobiological effects of MWCF is doubtful, because it was found absent in a cocktail of various distinct mono-, di-, and trimeric muropeptides possessing molecular mass ≤ 3 kDa that were detected after the digestion of PGN from *L. casei*13. Like MDP, other types of muropeptides have been found to possess immunostimulatory properties14,15. Another partial dipeptide structure of PGNs is gamma-D-glutamyl-meso-diaminopimelic acid which possesses weak immunobiological potential12,16. Furthermore, lactobacilli produce large and nopimelic acid which possesses weak immunobiological potential12,16. Furthermore, lactobacilli produce large and small muropeptides, ranging in MW from 3.48 to 2.041 kDa (ref.17). Although no reliable information on their immunomodulatory effects is available, some of them have been found associated with increased expression of TNF-α (ref.18).

In conclusion, the present results provide unequivocal evidence showing that bacterial lysates contain chemical entities with MW < 100 kDa possessing immunostimulatory properties. They are present in both G+ and G− bacteria. The low MW molecules, ≤ 3 kDa, are obviously the major contributors to the overall effect of lysates. Their share can be estimated to be approximately 55-85%, in dependence on the response and the type of bacteria. The findings warrant further studies which should identify chemical species and determine quantitative aspects of their occurrence in samples of different bacterial origin.

**ABBREVIATIONS**

IL-1β, interleukin-1β; LPS, lipopolysaccharide; LTA, lipoteichoic acid; MDP, muramyl dipeptide; MW, molecular weight; MWCF, molecular weight cutoff microfiltration fraction(s); NO, nitric oxide; PGN, peptidoglycan(s); TNF-α, tumor necrosis factor-α.

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INTRODUCTION

Anthocyanins (Greek anthos, flower and kyanos, blue) are water soluble natural pigments which belong to a large group of polyphenolics named flavonoids⁴. They are widely distributed in fruits and vegetables such as blueberries, cranberries, strawberries, cherries, plums, grapes, and red cabbage but are also found in flowers and other plant materials. Anthocyanins are responsible for cyanic colors ranging from salmon pink through red and violet to dark blue. There have been over 600 anthocyanins identified in nature, featuring six common aglycones – anthocyanidins (cyanidin, delphinidin, malvidin, peonidin, petunidin, pelargonidin). Their usual distribution in fruits² is: cyanidin 30%, delphinidin 22%, pelargonidin 18%, peonidin 7.5%, malvidin 7.5% and petunidin 5% . Daily dietary intake of anthocyanins has been estimated at 82 mg/day in Finland and 12.5 mg per day per person in the United States and can rise several fold depending on seasonal and lifestyle factors¹. Thus, their intake and also bioavailability are important in estimating beneficial effects of anthocyanins on human health.

Chemically, anthocyanins are derivatives of 2-phenylbenzopyrylium (flavylium cation) (Fig. 1). The anthocyanins consist of an aglycone (anthocyanidin), sugar(s), and, in many cases, acyl group(s). Sugars may be present as mono-, di- or trisaccharides. Glucose, galactose and arabinose are the sugars most commonly present in anthocyanins. The most common naturally occurring anthocyanins are the 3-O-glucosides and the 3,5-O-diglucosides of malvidin, cyanidin, pelargonidin, delphinidin, petunidin and peonidin⁶.

![Fig. 1. Structure of anthocyanins tested.](image)

Anthocyanins have a variety of physiological functions. Consumption of anthocyanins in fruits, vegetables, wines, jams, and preserves is associated with probable reduced risks of chronic diseases such as cancer, cardiovascular diseases, type II diabetes, virus inhibition, Alzheimer’s disease⁵. Anthocyanins and other flavonoids are regarded as important nutraceuticals mainly due to their antioxidant effects, which give them a potential role...
in prevention of the various diseases associated with oxidative stress.

The bioavailability of the anthocyanins is a main factor for their physiological functions. Anthocyanins are either rapidly absorbed from the gastrointestinal tract by different mechanisms that may involve specific enzymes such as bili translocases, or are first metabolized by the gut microbiota, leading to anthocyanidins and phenolic acid metabolites. However, several studies have demonstrated that anthocyanins are very poorly absorbed and are excreted only to a small extent in urine. In feeding studies with animals and humans, typically ca. 0.1% of the quantities ingested, and sometimes much less, has been detected in urine. Anthocyanins also undergo metabolism, since part have been found as glucuronidated, methylated or sulphated forms. This anthocyanin metabolites are delivered again to the gastrointestinal tract via enterohepatic circulation and after reaching the colon are subjected to bacterial metabolism by colon flora.

The available data imply that the determinants of absorption and excretion are influenced not only by the nature of the sugar moiety but also by the structure of the anthocyanidin aglycone. The complex array of information on anthocyanin bioavailability obtained with human and animal test systems has been reviewed by Prior and Wu.

Cytochrome P450 enzymes (P450s, or CYPs) are primarily responsible for most of the drug biotransformations in variety of tissues such as liver, kidney, brain, lung, and heart. Most are found in the endoplasmic reticulum, but five are localized primarily in mitochondria. P450s are involved in the detoxification of a wide variety of xenobiotics such as drugs, biogenic amines from food sources, environmental toxins, and chemical carcinogens, the oxidation of steroids, fatty acids, prostaglandins, leukotrienes, and fat-soluble vitamins.

The current study was undertaken to investigate an influence of anthocyanins on enzymatic activity of three human liver microsomal CYP forms important for metabolism of drugs and other xenobiotics.

MATERIALS AND METHODS

Chemicals
Cyanidin-3-O-glucoside chloride (kuromanin chloride), cyanidin-3-O-galactoside chloride (idein chloride), cyanidin-3-O-arabinoside, cyanidin-3,5-O-diglucoside chloride (cyanin chloride), delphinidin-3-O-glucoside (myrtillin chloride), malvidin-3-O-glucoside chloride (oenin chloride), peonidin-3-O-glucoside chloride, pelargonidin-3-O-rutinoside chloride, petunidin-3-O-glucoside chloride were purchased from Extrasynthese (Genay, France). All of tested compounds were dissolved in distilled water. All other chemicals were supplied by Sigma Aldrich (Prague).

Enzyme and substrates
Cryopreserved human liver microsomes (pooled) were purchased of Advancell (Barcelona, Spain). Microsomes were obtained under approval of the local ethics committee and in accordance with the ethic regulations of the country of origin (Spain). They were from five males and five females with a protein content of 38.4 mg/mL.

For determination of CYP activities, testosterone and diclofenac were supplied by Sigma Aldrich (Prague, Czech Republic), ethoxyresorufin was supplied by Fluka (Buchs, Switzerland) and 6β-hydroxytestosterone, 4-hydroxydiclofenac and 7-ethoxyresorufin were supplied by Cerilliant Corporation (Round Rock, Texas, USA).

Methods
Activities of individual CYP forms were measured according to published protocols. The following microsomal CYP activities were tested: CYP3A4, testosterone 6β-hydroxylation; CYP1A2, 7-ethoxyresorufin O-deethylolation; and CYP2C9 activity, diclofenac 4’-hydroxylation. Final incubation volumes were as follows: CYP1A2, 100 μL; CYP2C9, 200 μL nad CYP3A4, 500 μL. The reaction mixtures of all CYP activities tested were buffered by 100 mM K-phosphate buffer (pH 7.4). For each enzyme assay, a preliminary experiment was done to determine the Km and Vmax for given enzyme reaction and to obtain the values of substrate concentrations suitable for the inhibition experiments. Inhibition experiments were routinely performed with six concentrations of the tested compound (0, 10, 20, 40, 80 and 100 μM in the reaction mixture). The effects of anthocyanins on CYPs activity were determined using the Prominence system (Shimadzu, Kyoto, Japan).

RESULTS AND DISCUSSION
Possible interactions of nine anthocyanins (cyanidin-3-glucoside, cyanidin-3-galactoside, cyanidin-3-arabinoside, cyanidin-3,5-di-glucoside, delphinidin-3-glucoside, malvidin-3-glucoside, peonidin-3-glucoside, petunidin-3-glucoside and pelargonidin-3-rutinoside) with activities of three forms of P450 enzymes present in human liver microsomes (CYP3A4, CYP1A2 and CYP2C9) were studied.

In general, all the anthocyanins tested inhibited activities of CYP enzymes in a concentration-dependent manner (Fig. 2). The most prominent inhibition was observed with CYP3A4 activity (testosterone 6β-hydroxylation) by almost all anthocyanins; namely, with delphinidin-3-glucoside and cyanidin-3-arabinoside (down to 50% of control values) and malvidin-3-glucosides as well as cyanidin-3-glucosides (down to 60% of original values) at the highest concentration of tested compounds (100 μM). The only anthocyanins which did not inhibited the CYP3A4 enzyme activity by more than 20% were pelargonidin-3-rutinoside and cyanidin-3,5-diglucoside (Fig. 2). Inhibition of CYP2C9 enzyme activity, diclofenac 4’-hydroxylation, was more prominent with delphinidin-3-glucoside and cyanidin-3-galactoside (down to about 60% of the original activity at 100 μM concentration); other anthocyanins were weaker inhibitors of CYP2C9.
Fig. 2. Inhibition of CYP activities in human liver microsomes by different anthocyanins (cyanidin-3-glucoside, CY3GL; cyanidin-3-galactoside, CY3GA; cyanidin-3-arabinoside, CY3AR; malvidin-3-glucoside, MA3GL; peonidin-3-glucoside, PN3GL; petunidin-3-glucoside, PT3GL; delphinidin-3-glucoside, DL3GL; pelargonidin-3-rutinoside, PG3RU; cyanidin-3,5-di-glucoside, CY3,5DIGLU). Anthocyanin concentrations 0, 10, 20, 40, 80 and 100 μmol/L.
activity (activity decreased by 30 to 20%) (Fig. 2). Pelargonidin-3-rutinoside did not inhibit the CYP2C9 enzyme at all.

Activity of the CYP1A2 form (7-ethoxyresorufin O-deethylation) was affected by anthocyanins only slightly (activity decreased about 10%) with the exception of malvidin-3-glucoside exhibiting inhibition of CYP2C9 activity by 30% (Fig. 2).

The results indicate that – taking into account the relatively low bioavailability of anthocyanins in the plasma10 – the interactions of anthocyanins with liver CYP enzymes most probably does not pose any harm to human organism based on drug interactions. However, in case of a drug metabolized by CYP3A4 with a simultaneous ingestion of large amounts of anthocyanins, a limited increase of bioavailability of concomitantly taken drug may be observed which probably would not be also clinically significant.

ACKNOWLEDGMENT

The financial support from the Grant Agency of the Czech Republic (project P303/12/G163) and from the Palacky University (project LF_2013_007) is gratefully acknowledged.

CONFLICT OF INTEREST STATEMENT

Author’s conflict of interest disclosure: The authors stated that there are no conflicts of interest regarding the publication of this article.

REFERENCES

Silybin affects the liver microsomal CYP2C6 in HHTg rats

Rostislav Vecera, Alice Zacharová, Ludmila Kazdová, Zuzana Matusková, Nina Skottová, Jan Strojl, Olena Oliyarnyk, Pavel Anzenbacher

INTRODUCTION

Silymarin, a standardized extract from the seeds of the plant Silybum marianum (Milk Thistle), has been used in the supportive therapy of liver diseases for centuries. Silymarin has been promoted as a nutritional supplement for healthy liver function. Its cytoprotective effect is believed to be based on antioxidant properties. In our previous studies, we have shown a hypolipidemic effect of silymarin. Silybin is the main component of silymarin representing about 50% of this extract. This flavonolignan has antioxidant, anti-inflammatory, anti-viral, anti-fibrotic, anti-cancer, metabolic, and other beneficial effects. Given that silybin is taken by a lot of patients with metabolic syndrome and liver lipid accumulation, we were interested in its effects on the cytochrome P450 in terms of possible interactions with concomitantly administered medications. The risk and severity of drug-drug interactions generally rises with increasing dose of the drug and/or in concomitant administration with drug that may interact with the same transport or metabolic pathway. It should be noted that these drug-drug interactions present a leading cause of patient hospitalization and also death. CYP2C6 is one of the main enzymes of the cytochrome P450 (about 20% of the total CYP content) in rat liver microsomes. This cytochrome P450 can also be regarded as a counterpart of human CYP2C9. Rat CYP2C6 and human CYP2C9 are known to share substrates (both catalyze the 7-hydroxylation of warfarin and 4'-hydroxylation of diclofenac). High significance of human CYP2C9 in metabolism of many drugs (ibuprofen, warfarin or diclofenac) is well documented.

This study aims at investigating the effect of silybin (0.5% w/w), administered in high cholesterol and high fat diet for 3 weeks, on the expression of liver CYP2C6 in hereditary hypertriglyceridemic male Wistar (HHTg) rats. These animals selected from Wistar rats exhibit hypertriglyceridemia, liver steatosis, insulin resistance, and steatosis. This study aims at investigating the effect of silybin on cytochrome P450 2C6 expression in the liver of HHTg rats. Experimental animals fed the same diet as the controls, but with silybin (0.5% w/w), were also included in this experiment. The results obtained in this study are a very promising approach towards the use of silybin as an alternative treatment for metabolic syndrome.

MATERIAL AND METHODS

Experimental animals

Adult male hereditary hypertriglyceridemic Wistar (HHTg) rats (260–280 g of body weight, seven rats in each group) were maintained under standard laboratory conditions. The animals were fed (ad libitum): 1) standard laboratory diet (=STD), 2) high cholesterol diet (HCD), composed of STD + 1% of cholesterol w/w and 10% of lard fat w/w.
Table 1. Dietary intakes of standard laboratory diet (STD), high cholesterol diet (HCD) and HCD with 0.5% of silybin (HCD+0.5%SB).

<table>
<thead>
<tr>
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<th>HCD</th>
<th>HCD+0.5%SB</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MDC of diet</strong> (g.kg⁻¹ of body weight)</td>
<td>86.2±3.67</td>
<td>77.8±3.71</td>
<td>79.1±2.64</td>
</tr>
<tr>
<td><strong>MDD of drug</strong> (mg.kg⁻¹ of body weight)</td>
<td>-</td>
<td>-</td>
<td>395.5±8.4</td>
</tr>
<tr>
<td><strong>Body weight (g)</strong></td>
<td>340.1±5.5</td>
<td>348.3±6.2</td>
<td>345.3±4.5</td>
</tr>
</tbody>
</table>

Values are means ± SE, n=7; MDC – mean daily consumption of the diet, MDD – mean daily dose of silybin.

Fig. 1. Expression of CYP2C6 mRNA in liver of HHTg rats. Standard laboratory diet (STD), high cholesterol diet (HCD) and HCD with silybin (HCD + 0.5% SB). Values are means ± SE, n=7; **P<0.02 vs HCD, ++P<0.02 vs HCD.

Fig. 2. Activity of CYP2C6 in liver of HHTg rats. Standard laboratory diet (STD), high cholesterol diet (HCD) and HCD with silybin (HCD + 0.5% SB). Values are means ± SE, n=7; ***P<0.01 vs HCD.
**Expression of CYP2C6 protein**

![Graph showing expression of CYP2C6 protein](image)

**Fig. 3.** Expression of CYP2C6 protein in liver of HHTg rats. Standard laboratory diet (STD), high cholesterol diet (HCD) and HCD with silybin (HCD + 0.5% SB). Values are means ± SE, n=7.

10% of lard fat and 3) HCD with 0.5% (w/w) of silybin. After 21 days of feeding, the rats were fasted overnight. Animals were anesthetized by intramuscular administration of dexmedetomidin (200 μg.kg-1 of body weight) in combination with fentanyl (40 μg.kg-1 of body weight), followed by administration of diazepam (5 mg.kg-1 of body weight). Blood was sampled into EDTA tubes from the aortic bifurcation. Plasma was separated by centrifugation (2500 × g, 20 min, 4 °C). Rat liver was removed, rinsed in ice-cold sucrose solution, and frozen in dry ice. All procedures with animals were approved by the Ethics Committee, Ministry of Education, Czech Republic.

**Real-time PCR procedures**

A piece of liver tissue sample stabilized in RNA-later (Quiagen, Germantown, USA) was homogenized and subsequently passed through QIAshredder columns to eliminate tissue microparticles. RNA was isolated by RNeasy Plus Minikit (Quiagen Germantown, USA) which enable degradation of contaminating genomic DNA. 1 μg of RNA was reverse-transcribed by Transcriptor High Fidelity cDNA Synthesis Kit (Roche, Basel, Switzerland) and random hexamer primers. Thusly synthesized cDNA was utilized for RT-PCR with the use of a Light Cycler 480 SYBR Green Master I mix in a Light Cycler 480 (Roche, Basel, Switzerland) and random hexamer primers. The thermal cycling conditions were: 10 min at 95 °C, followed by 45 cycles at 95 °C for 10 s, at 58 °C for 15 s, and at 72 °C for 15 s for denaturation, annealing, and elongation, respectively. All samples for RT-PCR were prepared in triplicates. Fluorescence emission (cycle-to-cycle) was monitored and absolute quantification method was applied to obtain gene expression data. The respective rat primers were designed in our laboratory and consecutively synthesized by Invitrogen (Life Technologies, Prague, Czech Republic). The following primer sequences were used:

- CYP2C6 Fw 5'-GCCTTGTGGAGGAACTGAGG-3'
- CYP2C6 Rev 5'-GCAAGCCACAGGATAACGT-3'

**Activity of CYP2C6**

Formation of diclofenac metabolite (4′-hydroxy-diclofenac, formed mainly by CYP2C6) as well as the levels of diclofenac (standards from Sigma-Aldrich, Prague, Czech Republic) were determined in samples using a method by Crespi. Analyses were performed using HPLC with UV detection set at 280 nm (Shimadzu Prominence System, Kyoto, Japan). The metabolite was separated using LiChrospher 100 RP-18 column (4 x 250 mm ID) with a 5 μ particle size (Merck Millipore, Darmstadt, Germany) protected by LiChrospher 100 RP-18 precolumn (4 x 4 mm ID) of the same origin. 4′-hydroxydiclofenac was separated at 50 °C and at a flow 1 ml/min of mobile phase using gradient elution. The mobile phase A consisted of 2 mM perchloric acid in a water: acetonitrile (7:3, v/v) and the mobile phase B consisted of 100% methanol. The gradient steps were: 0-20 min linear gradient from 70% to 0% A and from 30% to 100% B; 20-22 min isocratic at 0% A and 100% B; 22-25 min gradient from 0% to 70% A, and from 100% to 30% B; 23-33 min isocratic at 70% A and 30% B.

**Western blot procedures**

Liver microsomes were obtained using routine procedure by Lake. Protein concentration was measured (BCA Protein Assay Reagent Kit, Pierce, Rockford, USA). 12.5 μg of protein from each sample was mixed with buffer (62.5 mM TRIS, 10% glycerol, 4% mercaptoethanol, 2% SDS, pH 6.8) in a volume ratio of 1:1 and electrophoresed (discontinuous SDS-polyacrylamide gel; 4% w/w stacking gel, 8% w/w separating gel). Following electrophoresis, proteins were transferred onto polyvinylidene difluoride membrane, Hybond-P (Amersham Biosciences, Piscataway, USA). Immunoreactivity was detected after 60 min incubation with a primary monoclonal CYP2C6 antibody (Abcam, Cambridge, UK) and 60 min incubation with a secondary antibody anti-mouse IgG - HRP.
conjugate (Sigma-Aldrich, Prague, Czech Republic). The reaction was detected using enhanced chemiluminescence (the manufacturer’s protocol, WB Luminol Reagent, Santa Cruz, USA). The blots were then exposed to medical X-Ray film and scanned (CanoScan Toolbox software, ver. 5.0.).

**Statistical analysis**

All data are expressed as means ± SE. (n=7). Differences between groups were analysed (analysis of variance, ANOVA) followed by the appropriate post-hoc test. Significance threshold was \( P<0.05 \). Western blot were analysed by ElfoMan software (ver. 2.6, Semecky Inc., Prague, Czech Republic).

**RESULTS**

The quantity of feed consumed (standard diet, high-cholesterol diet, and high-cholesterol diet with silybin) was checked daily per each cage holding one or two animals. The silybin dose (about 390 mg per kg of b.w. per day) was chosen in agreement with information published in literature\(^{22}\). As shown in Table 1, mean daily dietary intake and body weights of rats were not significantly different between any of the experimental groups.

HCD diet alone decreased mRNA expression of hepatic CYP2C6 (Fig. 1). Silybin supplementation of high cholesterol and high fat diet affected studied cytochrome P450. Unlike the HCD diet alone, silybin in this type of experimental diet significantly increased expression of CYP2C6 at the mRNA level to the level found in control group (Fig. 1). On the other hand, at the level of protein, no significant changes were observed (Fig. 3). Fig. 2 shows the effects of silybin on hydroxylation activity of CYP2C6. In hereditary hypertriglyceridemic rats fed the high cholesterol diet, silybin significantly increased the diclofenac hydroxylating (4’-hydroxylation) activity corresponding to the CYP2C6 enzyme activity\(^ {20}\). It is in good agreement with significant augmentation of its mRNA expression.

**DISCUSSION**

Our study shows that administration of the flavonolignan silybin to hereditary hypertriglyceridemic rats fed a high cholesterol diet results in an increase in the expression of mRNA of liver microsomal CYP2C6 enzyme. Positive effect of silybin on CYP2C6 has been confirmed by a significant rise of its enzymatic activity (is diclofenec 4’-hydroxylating\(^ {20}\)). This cytochrome P450 is considered to be a counterpart to human CYP2C9 (ref.\(^ {12}\)).

The enzyme activity and sensitivity of CYP2C6 mRNA expression to silybin is reported in hereditary hypertriglyceridemic rats (an accepted model of metabolic syndrome) for the first time. Publications often reports that silybin

**CONCLUSION**

Our results demonstrate that silybin significantly increased activity and mRNA expression of CYP2C6 in HHTg rats. It may be suggested that silybin has the potential to influence levels of drugs metabolized by rat CYP2C6, especially under conditions associated with metabolic syndrome and ectopic lipid accumulation in liver. However, the clinical relevance of studies performed on experimental animal models is not straightforward. Further studies are needed to elucidate the effects of silybin on CYP2C9 enzyme in man and also on other pleiotropic pathways, including the effect of diet and pathological state in humans.

**ABBREVIATIONS**

ANOVA, analysis of variance; BCA, biocinchoninic acid; cDNA, complementary deoxyribonucleic acid; CYP, cytochrome P450; HHTg, hereditary hypertriglyceridemia; HRP, horseradish peroxidase; mRNA, messenger ribonucleic acid; RT-PCR, real-time polymerase chain reaction; SDS, sodium dodecyl sulfate.
ACKNOWLEDGEMENTS
This work was supported by Czech Science Foundation project 13-10813S.

CONFLICT OF INTEREST STATEMENT
Author’s conflict of interest disclosure: The authors stated that there are no conflicts of interest regarding the publication of this article.

REFERENCES
INTRODUCTION

TAA is a thiono-sulfur containing compound that has been studied and used as a highly specific hepatotoxic substance both in vitro and (more frequently) in vivo. Single dose can induce acute hepatic failure, while chronic oral or intraperitoneal administrations of TAA are established methods in the generation of fibrosis and cirrhosis models in rats. TAA is used for its highly specific hepatotoxic effect and the ability to produce liver damage with histological appearance similar to human hepatic fibrosis.

TAA produces centrilobular necrosis and the cytotoxic effect is mediated by its metabolites, not the compound itself. TAA is rapidly oxidized by hepatic microsomal CYP2E1 to acetamide and thioacetamide sulfoxide which is further metabolized to thioacetamide-S,S-dioxide. This highly reactive metabolite binds covalently to intracellular macromolecules and can initiate necrosis and cause oxidative stress. Bioactivation of TAA and its active metabolite follows saturable kinetics. It was shown that the generation of reactive oxygen species plays an important role in TAA-induced liver damage and the development of fibrosis. The ability of antioxidant substances to inhibit or attenuate the progression of thioacetamide induced liver damage and to decrease its prooxidant effects has been proven in many studies.

The aim of this preliminary experiment was to study the time course of activities of ALT, AST and GLDH in serum and markers of oxidative stress in the liver and kidneys of rats after chronic TAA administration.

RESULTS

Significant increases in serum ALT and GLDH persisted for 16 weeks after the treatment, while serum AST was increased only in animals sacrificed 4 weeks after the treatment cessation. No increase in LP or decrease in GSH was observed in the liver. Furthermore, a decrease in LP and an increase in GR activity appeared in the 16th week. Significant decreases in the activities of catalase and GPx (which persisted in animals sacrificed 12 and 16 weeks after the treatment, respectively) were the only markers of hepatic oxidative damage. In kidneys, LP was significantly increased 4 and 12 weeks after the TAA treatment which implies the importance of oxidative stress in the renal damage that develops as a consequence of liver cirrhosis.

Key words: thioacetamide, liver, kidney, oxidative stress, aminotransferases, rat

INTRODUCTION

TAA is a thiono-sulfur containing compound that has been studied and used as a highly specific hepatotoxic substance both in vitro and (more frequently) in vivo. Single dose can induce acute hepatic failure, while chronic oral or intraperitoneal administrations of TAA are established methods in the generation of fibrosis and cirrhosis models in rats. TAA is used for its highly specific hepatotoxic effect and the ability to produce liver damage with histological appearance similar to human hepatic fibrosis.

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The aim of this preliminary experiment was to study the time course of activities of ALT, AST and GLDH in serum and markers of oxidative stress in the liver and kidneys of rats during the period of 16 weeks after chronic TAA administration. It has been shown that administration of low intraperitoneal or oral doses of TAA for 8-14 weeks can induce liver injury and dysfunction and cause hepatic fibrosis in mice and rats.

MATERIALS AND METHODS

Chemicals

Thioacetamide and other reagents were of analytical grade and obtained from Sigma-Aldrich (USA) or as indicated in the specific methods.

Animals and treatment

Female Wistar rats (7-8 weeks of age) were housed at standard laboratory conditions under controlled temperature (22±2 °C), relative humidity (50-60%), and 12-h light-dark cycles. The animals had free access to water and standard pellet rat chow. After 11 days of acclimatization, forty-six rats were randomly divided into two
groups: Animals in the treated group were given TAA (intraperitoneally 200 mg/kg body weight) three times per week over a period of 12 weeks. The dose was based upon literature. Animals in the control group received saline.

Animals were sacrificed 4, 12 or 16 weeks after the discontinuation of the treatment. Blood and tissue samples were collected and used immediately or stored frozen at -70°C until analyzed. Lipid peroxidation (LP), reduced glutathione (GSH) and activities of glutathione peroxidase (GPx), glutathione reductase (GR) and catalase (CAT) were estimated in liver and kidney homogenates. Activities of alanine amino transferase (ALT), aspartate amino transferase (AST) and glutamate dehydrogenase (GLDH) were determined in serum. TAA treated animals were compared with age-matched controls.

The experimental treatment protocol was approved by the local Animal Care and Use Committee.

Table 1. Lipid peroxidation (LP), reduced glutathione (GSH), and activities of glutathione peroxidase (GPx), glutathione reductase (GR) and catalase (CAT) in the liver of thioacetamide-treated rats sacrificed 4, 12 and 16 weeks after the discontinuation of the treatment.

<table>
<thead>
<tr>
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<th>Liver</th>
<th>4 weeks</th>
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<tr>
<td></td>
<td></td>
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<tr>
<td></td>
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</tr>
</tbody>
</table>

Values are means ±SD; ***P<0.001, **P<0.01 and *P<0.05 vs control group.

Table 2. Lipid peroxidation (LP), reduced glutathione (GSH), and activities of glutathione peroxidase (GPx), glutathione reductase (GR) and catalase (CAT) in kidneys of thioacetamide-treated rats sacrificed 4, 12 and 16 weeks after the discontinuation of the treatment.

<table>
<thead>
<tr>
<th></th>
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<td>TAA</td>
<td>107.1±12.5***</td>
<td>99.1±9.5**</td>
<td>96.5±6.8</td>
</tr>
<tr>
<td></td>
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<td></td>
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<tr>
<td></td>
<td>control</td>
<td>4.62±0.34</td>
<td>4.61±0.36</td>
<td>4.74±0.31</td>
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<td></td>
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<td>4.68±0.33</td>
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<td></td>
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<tr>
<td></td>
<td>control</td>
<td>9.4±0.8</td>
<td>9.0±0.3</td>
<td>10.3±0.8</td>
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<td></td>
<td>TAA</td>
<td>10.1±1.0</td>
<td>10.7±1.0***</td>
<td>11.1±0.9</td>
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<tr>
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<tr>
<td></td>
<td>control</td>
<td>7.43±0.27</td>
<td>5.92±0.46</td>
<td>7.82±0.59</td>
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<tr>
<td></td>
<td>TAA</td>
<td>7.39±0.39</td>
<td>6.40±0.57</td>
<td>7.42±0.63</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>9.8±0.6</td>
<td>10.8±1.2</td>
<td>9.9±0.9</td>
</tr>
<tr>
<td></td>
<td>TAA</td>
<td>9.7±0.6</td>
<td>12.1±1.4*</td>
<td>11.5±1.6*</td>
</tr>
</tbody>
</table>

Values are means ±SD; ***P<0.001, **P<0.01 and *P<0.05 vs control group.
Biochemical assays

Lipid peroxidation (LP) was estimated in liver and kidney homogenates by measuring the products formed in the thiobarbituric acid (TBA) reaction\textsuperscript{26}. Tissue homogenates (0.25 g/2.5 mL of 1.15% potassium chloride (KCl)) were mixed with 1.5 mL of 1% phosphoric acid (H\textsubscript{3}PO\textsubscript{4}) and 0.5 mL of 0.6% TBA aqueous solution. The samples were heated at 95 °C for one hour after cooling, 2 mL of n-butanol were added, mixed vigorously and the butanol phase was separated by centrifugation. The absorbance of butanol layer was measured at 520 and 535 nm; the difference between the determinations was used to calculate concentration of TBA reactive substances (TBARS). The results are expressed in nmole TBARS/gram of tissue.

GSH level was estimated in the deproteinized supernatant fraction of liver and kidney homogenates (0.2 g/8 mL of 0.02 M EDTA) using 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB, Ellman’s reagent) and reading absorbance at 412 nm (ref.\textsuperscript{27}). The results are expressed in μmol GSH/g of tissue.

GPx activity was assayed in liver and kidney homogenates by a coupled test system, in which GR is employed for the regeneration of reduced glutathione and butyl hydroperoxide used as the acceptor substrate\textsuperscript{28}. The decrease in NADPH concentration was registered photometrically at 340 nm. The GPx activity is expressed in μmol NADP/minute/g of tissue.

GR assay is based on the reduction of oxidized glutathione (GSSG) by NADPH in the presence of glutathione reductase. The formed GSH reacts with 5,5'-dithiobis(2-nitrobenzoic acid). The increase in absorbance at 412 nm was measured\textsuperscript{29}. The reaction system contained 0.1M phosphate buffer (pH 7.5), 1mM EDTA, 2mM GSSG and 3mM DTNB solution. Reactions were started by the addition of 2mM NADPH and the increase in absorbance was measured at 412 nm.

Catalase activity was estimated according to the method of Aebi et al.\textsuperscript{30} by following the decomposition of H\textsubscript{2}O\textsubscript{2} directly by the decrease in extinction of hydrogen peroxide at 240 nm. The activity of catalase is expressed as a rate constant of a first order reaction k per g of tissue.

The serum activity of ALT, AST and GLDH were estimated photometrically using the commercial kits (DiaSys Diagnostic System, Germany), according to the manufacturers’ protocol.

Statistical analysis

The results are expressed as means ± SD. Significant differences between experimental groups were estimated using unpaired Student’s t test (GraphPad InStat3). Differences between the groups were considered significant at $P<0.05$.

RESULTS

TAA administration significantly increased serum ALT, AST and GLDH activities. The increase in activi-
ties of ALT and GLDH in TAA-treated rats (Fig. 1, Fig. 3) persisted for 16 weeks following the treatment cessation, increased AST activity was detected only in TAA-treated animals sacrificed 4 weeks after the treatment discontinuation and returned to control levels in animals sacrificed later (12 and 16 weeks after the treatment) (Fig. 2).

No increase in hepatic LP was observed. Contrary to the assumption, a decrease in LP and an increase in GR activity even appeared in the liver of TAA-treated rats in the 16th week following the treatment cessation. Significant decreases in the activity of GPx, which persisted for 16 weeks after the TAA treatment and in the activity of catalase, which was seen in rats sacrificed 4 and 12 weeks after the treatment, were the only markers of oxidative damage in the liver. We have not detected any changes in the content of hepatic GSH (Table 1).

Significant increase in LP was found in kidneys of rats which were sacrificed 4 and 12 weeks after the treatment cessation. Contrarily, a significant increase in the activity of GPx in kidneys (in animals sacrificed 12 weeks after the treatment) and in the activity of catalase in kidneys (in animals sacrificed 12 and 16 weeks after the treatment) was also detected. There were no changes in kidney GSH content (Table 2.).

DISCUSSION

Chronic administration of TAA over the period of 12 weeks induced liver damage that was characterized by a significant increase in serum activities of ALT and GLDH that persisted for 16 weeks following the treatment discontinuation. The activity of AST was significantly increased only in the animals sacrificed in the 4th week after the treatment. This might reflect the possible tissue repair or cessation of injury progression, since the mitochondrial AST is released only when the cells are severely disintegrated.

Oxidative stress has been proven to be involved in the pathogenesis of TAA induced liver damage and a depletion of hepatic GSH and an increase in TBARS concentration are typical markers of oxidative stress seen during both acute and chronic thioacetamide intoxication. In our study, we found neither an increase in LP nor a decrease in GSH content in the liver during the period from the 4th to the 16th week after the cessation of TAA treatment. However, we have detected some markers of deterioration of hepatic oxidative state since the activity of catalase was significantly decreased in animals sacrificed 4 and 12 weeks after the end of the treatment and the activity of GPx remained decreased even in the 16th week after the treatment discontinuation. Contrary to our expectations, we saw a decrease in LP and an increase in GR activity in the liver in the 16th week after the treatment cessation for which the explanation is not clear at the moment.

In animals sacrificed 4 or 12 weeks after the discontinuation of TAA treatment, the increase in LP in kidneys was found. Development of liver cirrhosis can result in damage to various distal organs and the increased LP that was seen in the kidneys of rats in our study is consistent with the conjecture that the renal damage (as well as the impairment of other organs) developed as a consequence of cirrhosis is presumably mediated by oxidative stress rather than by a direct toxic effect of a hepatotoxic agent. This can be further confirmed by an increase in LP in heart that we have observed as well with some delay after chronic TAA administration (our not yet published findings). In the kidneys, we have also seen an increase in the activities of GPx and catalase. This discrepancy can be explained as a response of these antioxidant enzymes to the increase in LP that was detected in the kidneys. Such reaction of antioxidant enzymes has already been described.

ACKNOWLEDGEMENT

Supported by the grant Charles University Grant Agency - GAUK 99510, project CZ.1.05/2.1.00/03.0076 from European Regional Development Fund and by the Charles University Research Fund (project number P36).

CONFLICT OF INTEREST STATEMENT

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REFERENCES


CLINICAL PHARMACOLOGY
COMMUNICATIONS
C-5

Prevalence of thromboembolic complications in patients with atrial fibrillation in relation to a selected antithrombotic therapy

Veronika Muller Zavalova a,b, Vaclav Zizlavska, Robert Staffa a

Aim. The aim of this work was to find and characterize the correlation between the development of peripheral arterial thromboembolism and the selected medication for the atrial fibrillation-induced coagulopathy.

Methods. The evaluated set included 103 patients admitted to the 2nd Department of Surgery, St. Anne’s University Hospital in Brno, during a period of 9 months. Patients were divided into individual groups on the basis of chronic medication of antithrombotic drugs, and the therapy effectiveness was evaluated on the basis of the thromboembolia prevalence.

Results. In total, there were 36 thromboembolic complications; in 14 patients, it was a relapse. In the warfarin-administered group, thromboembolia occurred in 31.6% patients and in the acetylsalicylic acid (ASA)-administered group, in 24.4% of cases. The highest prevalence of peripheral arterial thromboembolism was observed in the group without any antithrombotic therapy, where this diagnosis was determined in 78.57% of cases. A significant correlation (P=0.004; OR=7.94; CI 99% 1.183-53.33) was confirmed between the manifestation of coagulopathic states in patients with anticoagulation therapy and unmedicated patients. The smallest incidence of these complications was observed in the group with antiplatelet medication (P=0.0004; OR=11.33; CI 99% 1.693-75.89) compared to unmedicated patients. In the case of warfarin, the pharmacotherapy failure was caused by an insufficiently effective INR, which reached on average 1.42 ± 0.53. Furthermore, a high impact of drug interactions cannot be definitely ruled out, especially in the case of antiplatelet ASA therapy, individually or in combination with clopidogrel.

Key words: atrial fibrillation, thromboembolism, anticoagulation, antiplatelet therapy

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INTRODUCTION

Atrial fibrillation is one of the most frequent clinically significant disorders of the heart rhythm. The prevalence of this disease has been growing, and correlates with an increasing polymorbidity, age and sex of patients. To date, the number of patients reaches about 2% of population1-3 and because this figure does not include asymptomatic arrhythmias, the actual number of patients with atrial fibrillation is probably much higher4-5. The main cause of the increased morbidity and mortality in these patients results from up to 6-fold higher risk of thromboembolic complications6-7, especially stroke as well as transient ischemic attacks, and embolism in peripheral lower limbs or the visceral artery. In clinical practice, atrial fibrillation is most often associated with the presence of a very serious form of stroke with serious neurological deficit, up to 23% 90 days mortality (after 2 years over 45%) and uncertain prognosis of patients6. Etiopathogenesis of thromboembolic complications is derived from hemodynamic changes and structural abnormalities of myocardium. Pharmacotherapy is in most patients with atrial fibrillation from the perspective of altering hemodynamic conditions. Optimally balanced antithrombotic pharmacotherapy takes into consideration age and sex of patients, heart failure, hypertension, diabetes, incidence of stroke/transitory ischemic attack/thromboembolic complications and existence of vascular disease in anamnesis. Identifying of these clinically significant prediction factors resulted in the creation of CHA2DS2-VASc scoring system6 (Table 1), which, based on scored evaluation of risks, allows individual therapeutic approach. Individual categories of a selected antithrombotic therapy are given by the sum of obtained score. When reaching the value ≥ 2, there is indicated anticoagulation therapy, whereas values 0-1 prefer antiplatelet or no therapy, depending on the benefits/risks evaluation for every patient. Score interval 1-2 characterizes an area, where can be indicated both antplatelet and anticoagulation medication. Optimum therapy is chosen with respect to other possible limits, which are not addressed by CHA2DS2-VASc (ref.8). The risk of hemorrhagic complications (GI tract, brain, urinary tract) significantly increases in connection with antithrombotic therapy9-11, and they can be predicted by stratification of anamnestic data using the HAS-BLED score. This system allows quantification of a potential hemorrhage risk due to the evidence of parameters such as hypertension, abnormal renal/liver function, stroke, bleeding history or...
predisposition, labile INR, elderly (≥65), drugs/alcohol concomitantly. The score 3 and more indicates a high risk of hemorrhage and based on this evaluation, it is necessary to consider some particular anticoagulation or antiplatelet therapy with an aim to effectively decrease the thromboembolism incidence.

The aim of this study is to compare the frequency of life-threatening thromboembolic complications in connection with a selected antithrombotic medication, and to find correlation between the treatment failure and possible causal factors.

**MATERIALS AND METHODS**

The evaluated set characterizes 103 patients with atrial fibrillation admitted to the 2nd Department of Surgery, St. Anne’s University Hospital in Brno, during a period of 9 months (September 2012 to May 2013), regardless the main diagnosis. Due to their main focus, there was monitored the incidence of thromboembolism in peripheral artery in patients included in this study. The necessary biochemical and clinical examination was carried out within 24 hours after a patient’s acceptance, and surgical intervention was scheduled with respect to the patient’s overall condition. The evaluated group of patients included 45 women and 58 men, average age 74 years (45-103) (Table 2), and no excluding criteria were applied. Uneven representation of patients in individual groups is caused by the actual number of patients treated in this department. In the monitored group, 4 patients died due to massive thromboembolic complications and there were 3 major amputation of the limb. Patients with atrial fibrillation in their anamnesis were divided into 4 groups; 3 groups were medicated various chronic antithrombotic pharmacotherapy (warfarin at 1.5 - 10 mg/dose, acetylsalicylic acid 100 mg + clopidogrel 75 mg/dose) and 1 group of patients without indicated antiplatelet/anticoagulation medication. Incidence of thromboembolic complications was quantified for individual groups and graphically processed by Excel® (Microsoft). Odds ratio was used to compare the

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**Table 1. Risk factor-based approach expressed as a point based scoring system CHA₂DS₂-VASc. Clinical characteristics of HAS-BLED bleeding risk score.**

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>score</th>
<th>Risk factor</th>
<th>score</th>
</tr>
</thead>
<tbody>
<tr>
<td>C Congestive heart failure/LV dysfunction</td>
<td>1</td>
<td>H Hypertension</td>
<td>1</td>
</tr>
<tr>
<td>H Hypertension</td>
<td>1</td>
<td>A Abnormal renal/liver function</td>
<td>1 or 2</td>
</tr>
<tr>
<td>A₂ Age &gt;75</td>
<td>2</td>
<td>S Stroke</td>
<td>1</td>
</tr>
<tr>
<td>D Diabetes mellitus</td>
<td>1</td>
<td>B Bleeding</td>
<td>1</td>
</tr>
<tr>
<td>S₂ Stroke/TIA/thromboembolism</td>
<td>2</td>
<td>L Labile INRs</td>
<td>1</td>
</tr>
<tr>
<td>V Vascular disease</td>
<td>1</td>
<td>E Elderly (e.g. age &gt;65 years)</td>
<td>1</td>
</tr>
<tr>
<td>A Age 65–74</td>
<td>1</td>
<td>D Drugs or alcohol (1 point each)</td>
<td>1 or 2</td>
</tr>
<tr>
<td>Sc Sex category (i.e. female sex)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum score</td>
<td>9</td>
<td>Maximum score</td>
<td>9</td>
</tr>
</tbody>
</table>

CHA₂DS₂-VASc; score = 1: no antithrombotic therapy recommended; score = 1 antithrombotic therapy with anticoagulation/antiplatelet therapy recommended; score ≥2 anticoagulation therapy recommended. HAS-BLED score ≥3 indicates the risk of bleeding, and regular clinical review is recommended.

**Table 2. Baseline characteristics of the participants, according to the treatment group. (W-warfarin, ASA-acetylsalicylic acid, CLO-clopidogrel)**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>W 1.5 - 10 mg/day</th>
<th>ASA 100 mg/day</th>
<th>ASA+CLO 100+75 mg/day</th>
<th>no medication</th>
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<td>Age - yr</td>
<td>73.1±6.96</td>
<td>73.8±9.02</td>
<td>79.0±7.95</td>
<td>77.6±12.56</td>
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<td>M - no./total no. (%)</td>
<td>24/38 (63.2 %)</td>
<td>27/45 (60.0 %)</td>
<td>4/6 (66.7 %)</td>
<td>3/14 (21.4 %)</td>
</tr>
<tr>
<td>F - no./total no. (%)</td>
<td>14/38 (36.8 %)</td>
<td>18/45 (40.0 %)</td>
<td>2/6 (33.3 %)</td>
<td>11/14 (78.6 %)</td>
</tr>
</tbody>
</table>
obtained results for individual groups, expressing the risk ratio of peripheral arterial thrombosis. Statistical analysis of data used Fisher’s exact probability test at statistical significance levels $P<0.01$ and $P<0.001$. GraphPad Prism 5.00 software (GraphPad Software, San Diego, CA, USA, www.graphpad.com) was used to evaluate the data.

RESULTS

Out of the total number of 103 patients with atrial fibrillation, thromboembolic complications occurred in 36 patients, i.e. 34.95% cases. In 14 of them, it was a relapse. The group with anticoagulation warfarin (W) therapy at 1.5-10 mg/day included 38 patients, where 12 patients, i.e. 31.58%, experienced arterial thrombosis (Fig. 1). The INR value obtained within 24 hours after the acceptance reached on average 1.42 ± 0.53. The second group with only antiplatelet therapy by ASA at 100 mg/day included 45 patients (Fig. 1). In this group, thromboembolic complications were observed in 11 patients, i.e. 24.44%. The group with dual antiplatelet by ASA at 100 mg/day combined with clopidogrel (CLO) at 75 mg/day, included only 6 patients, and thromboembolia occurred in 2 cases (33.33%). The highest prevalence of peripheral arterial thrombosis was observed in the group of patients without anticoagulation and antiplatelet therapy, where this diagnosis was revealed in 78.57% of cases. When compared with the warfarin group, these patients are at almost 8 fold higher risk of thromboembolia and the prevalence of this condition in the monitored patients was characterized at the statistical significance level ($P=0.004$; OR=7.94; CI 99% 1.183-53.33). In patients with chronic medication of ASA at 100 mg/day, there was observed the lowest frequency of thromboembolic complications ($P=0.0004$; OR=11.33; CI 99% 1.693-75.89), compared with the group without antithrombotic medication, where the risk of the peripheral arterial thrombosis is up to 11 fold higher.

DISCUSSION

Medication therapy of coagulopathy in atrial fibrillation has exactly defined recommendations complying with CHA$_2$DS$_2$-VASc score. Patients with the score 1-2 represent a specific group, where the expertise and experience of the physician and psychosocial habits of a patient have a significant impact on the choice of antithrombotic medication. The process of considering the risk of bleeding in comparison with thromboembolia in order to optimize the effective therapy requires maximum information. At present, warfarin therapy has been preferred despite numerous dietetic and drug interactions. It was also preferred due to the setting of predictive CHA$_2$DS$_2$-VASc evaluation score, where most patients were classified in the category of medium-serious and higher risk of thromboembolic complications with a consequent indication of anticoagulation therapy$^{14}$. Pharmacovigilance studies confirm up to 64% decrease in the incidence of stroke$^{15}$ in connection with a chronic use of warfarin, accompanied by a relatively small number of hemorrhagic complications$^{16}$. However, the success of this therapy is influenced by optimum therapeutic range INR 2.0-3.0, which was achieved in our study group in only 18% of patients. In other cases, INR value did not exceed on average 59.2% of the target range (71% - 47.3%). Despite this fact, the warfarin therapy seems advantageous in comparison with the group of unmedicated patients, and the prevalence of cardiovascular complications of thromboembolic nature is up to 8-fold lower. Antiplatelet therapy by ASA indicated in cases, when patients cannot be medicated by warfarin due to the negative benefits/risks ratio or if the present condition does not require anticoagulation therapy in particular. Even though the warfarin therapy represents a golden standard in the antithrombotic therapy, our results imply a higher efficiency of the ASA therapy (24.44% vs 31.58% in warfarin), which corresponds to 11 fold lower probability of the development of cardiovascular thromboembolism. Even though warfarin is a “gold standard” in
the antithrombotic therapy, our results imply a higher effectiveness of the ASA therapy (24.44% vs 31.58% in warfarin) in the cardioembolic prevention. This statement is also supported by the odds ratio in these two groups, where the incidence of arterial thromboses ratio reaches 11:8 (ASA:warfarin). Similar results have been reported in various clinical studies17. Analogy of this therapy with a more significant prophylactic value of stroke is characterized by dual antiplatelet (ASA+clopidogrel). This therapy brings a significant increase in the risk of hemorrhagic complications, reaching the incidence of up to 4.5% patients after a year of therapy18. The number of observed hemorrhages is quantified at similar frequency as in the case of warfarin19. In predisposed patients, i.e. patients with ulcerative gastroduodenal disease in their anamnesis, etc., the danger of such complication is much higher. They are therefore preventively administered proton pump inhibitors (PPI - omeprazole, lansoprazole, pantoprazole and esomeprazole). More and more clinical studies have implied the association between thromboembolic complications and co-medication of PPI and clopidogrel18,19, or of PPI and ASA in mono-therapy20. Due to the common co-medication by these drugs, a great emphasis has been given to the clinical research of their interactions. Nevertheless, the controversy in the obtained clinical and biochemical results does not implicitly prove the significance of the monitored interactions. Yet it was possible to correlate this combination with the failure of antiplatelet mono- (22.44%) and dual (33.33%) therapy in several patients included in our study group. At the same time, it is necessary to consider the small set of patients with the combined therapy of ASA and clopidogrel, which has a substantial impact on the statistical evaluation and insufficiently relevant data, which would not, with a great probability, in case of a larger set of patients, reach the frequency of 33.33% of the development of thromboembolia.

CONFLICT OF INTEREST STATEMENT

Authors’ conflict of interest disclosure: None declared.

REFERENCES


Pharmacogenomics of infliximab therapy, impact of TNFRSF1A and TNFRSF1B gene polymorphisms

Michal Kolorz, Katerina Wroblova, Jana Mokranova, Ladislava Bartosova, Petr Dite, Vladimir Zboril, Milan Bartos

INTRODUCTION

Biological therapy is today regarded as the most effective therapy in various diseases, which pathophysiology results from excessive immune activation and pathological prolongation of inflammatory reaction caused by an excessive activation of TNF-alpha. This is also confirmed by the clinical effect of monoclonal antibodies neutralizing TNF-alpha and preventing manifestation of its functions. Infliximab became the first drug from the group of anti-TNF antibodies, which was used in the CD therapy. However, despite all benefits of the infliximab therapy, there remain approximately 30% of refractory patients. Anti-TNF antibodies, which was used in the CD therapy.

A basic signaling mechanism at the molecular level is the interaction with TNFR (ref.11). Both TNF forms (soluble (sTNF) and membrane-bound (tmTNF)) have an affinity to both types of receptors. Both TNF forms influence cells with TNFR on the membrane and trigger signaling cascade, which results in apoptosis or activation of nuclear factor NF-κB and expression of pro-inflammatory genes. Activation of TNFR1 (coded by TNFRSF1A gene) results in the internalization of the ligand-receptor complex and association of the “death domain” with adaptor proteins present in the cell’s cytoplasm. In case that after the binding of TNF-alpha to TNFR1 there is no internalization of this complex, the intracellular signaling is led via the activation of NF-κB, nuclear factor, which influences the expression of several pro-inflammatory genes. Similar process also occurs through interaction of sTNF with TNFR2 (coded by TNFRSF1B gene) (ref.19). Thus TNFR1 could exert opposing biological effects - pro-apoptotic or pro-inflammatory, depending on the activation of caspase or NF-κB signalization, respectively. For TNFR2, there is a typical interaction with tmTNF constituting an inherent part of the TNF-alpha negative reversal regulation of expression. This provides for the system of reverse signaling, leading to the therapy, medical risks and associated high costs of biological treatment resulted in an intensive research of factors, which would allow selection of patients with the greatest chance for the therapeutic success. In the last decade, there has been an emphasis on the patient’s genetic profile, besides clinical parameters and biochemical markers of the disease. Even though the results have so far been inconsistent, the research intensively focuses on the area of genome containing genes for TNF-alpha and proteins, which participate in the signaling processes.

Method. A total of 116 Caucasian CD patients treated with infliximab were genotyped. After initial 10 weeks of the infliximab therapy, effectiveness was determined and patients were divided into responders (n=98) and non-responders (n=18). Genotypes TNFRSF1A (T4672G, G3794C) and TNFRSF1B (T11695C, T587G) were determined by PCR-RFLP.

Results. Frequencies of variant alleles of TNFRSF1A were comparable between responders and non-responders. Variant allele TNFRSF1B 11695C was more common in non-responders (41.7% vs. 30.1%). Similarly, the frequency of TNFRSF1B 587G allele in non-responders was 33.3% vs. 18.9% in responders. Homozygotes for variant alleles of TNFRSF1B 11695C were found more often (P=0.013; OR 5.89, CI 95% 1.6-22.1) in non-responders (n=5, 27.8%) than in responders (n=6, 6.1%). Our results imply that TNFRSF1B 11695C variant allele is associated with a low therapeutic effect of infliximab.

Key words: infliximab, single nucleotide polymorphism, pharmacogenomics, TNFR

Introduction. Anti-TNFα monoclonal antibodies present an effective way of treating Crohn’s disease (CD). Despite their high benefits, there is about 30% rate of a primary non-response. The main target of infliximab is the soluble form of TNFα, which blocks its pro-inflammatory activity and the induction of apoptosis via the TNFα membrane form. The activity of TNFα and balance between its pro-inflammatory and pro-apoptotic effect is mediated by the interaction with its receptors (TNFR). Mechanisms of signaling via TNFα-TNFR interaction has been recently intensively studied from a perspective of selecting appropriate candidates for the infliximab treatment.

Aim. The aim of this study was to evaluate whether polymorphisms in TNFRSF1A and TNFRSF1B genes influence the efficacy of the infliximab therapy.

Methods. A total of 116 Caucasian CD patients treated with infliximab were genotyped. After initial 10 weeks of the infliximab therapy, effectiveness was determined and patients were divided into responders (n=98) and non-responders (n=18). Genotypes TNFRSF1A (T4672G, G3794C) and TNFRSF1B (T11695C, T587G) were determined by PCR-RFLP.

Results. Frequencies of variant alleles of TNFRSF1A were comparable between responders and non-responders. Variant allele TNFRSF1B 11695C was more common in non-responders (41.7% vs. 30.1%). Similarly, the frequency of TNFRSF1B 587G allele in non-responders was 33.3% vs. 18.9% in responders. Homozygotes for variant alleles of TNFRSF1B 11695C were found more often (P=0.013; OR 5.89, CI 95% 1.6-22.1) in non-responders (n=5, 27.8%) than in responders (n=6, 6.1%). Our results imply that TNFRSF1B 11695C variant allele is associated with a low therapeutic effect of infliximab.

Key words: infliximab, single nucleotide polymorphism, pharmacogenomics, TNFR

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to an inhibition of cytokines synthesis and apoptosis of cells with tmTNF present at the membrane\(^\text{*}\).

In this work, we retrospectively focused on the possible correlation between the infliximab effect in patients with CD and the occurrence of polymorphisms present in the genes for *TNFRSF1A* and *1B*.

**MATERIAL AND METHODS**

**Patients**

In this retrospective study we genotyped 116 unrelated Caucasian (Czech and Slovak) CD patients that were recommended the biological therapy according to standard criteria. All patients were given informed consent before entering the study. The study was approved by The Ethics Committee of University Hospital Brno.

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### Table 1. Characteristics of the study population.

<table>
<thead>
<tr>
<th></th>
<th>n (%)</th>
<th>Age</th>
<th>M/F</th>
</tr>
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<tbody>
<tr>
<td>Study population</td>
<td>116</td>
<td>38.2 (22-69)</td>
<td>71/45</td>
</tr>
<tr>
<td>Responders</td>
<td>98 (84)</td>
<td>38.5 (22-69)</td>
<td>60/38</td>
</tr>
<tr>
<td>Clinical and morphological response</td>
<td>51 (44)</td>
<td>38.5 (22-69)</td>
<td>31/20</td>
</tr>
<tr>
<td>Clinical response</td>
<td>47 (41)</td>
<td>38.5 (24-63)</td>
<td>29/18</td>
</tr>
<tr>
<td>Non-responders</td>
<td>18 (16)</td>
<td>36.4 (25-65)</td>
<td>11/7</td>
</tr>
</tbody>
</table>

### Table 2. Clinical criteria for the evaluation of therapeutic effect.

<table>
<thead>
<tr>
<th>Clinical criteria</th>
<th>CDAI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary responders</strong></td>
<td></td>
</tr>
<tr>
<td>CMR</td>
<td>Symptomatologic improvement, endoscopically confirmed morphological healing</td>
</tr>
<tr>
<td>CR</td>
<td>Symptomatologic improvement</td>
</tr>
<tr>
<td><strong>Non-responders</strong></td>
<td></td>
</tr>
<tr>
<td>Resistance</td>
<td>No symptomatology improvement</td>
</tr>
</tbody>
</table>

CDAI- Crohn’s disease activity index; CMR-Clinical and morphological response; CR-Clinical response.

### Table 3. Genotype frequencies in study group, primary responders and non-responders.

<table>
<thead>
<tr>
<th></th>
<th>Primary responders (n=98)</th>
<th>Non-responders (n=18)</th>
<th>All (n=116)</th>
<th>MAF</th>
</tr>
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<tbody>
<tr>
<td><strong>TNFRSF1A</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-690G&gt;T</td>
<td>GG</td>
<td>38</td>
<td>18</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>GT</td>
<td>44</td>
<td>22</td>
<td>22</td>
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<td>5</td>
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<td>-1488G&gt;C</td>
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<td></td>
<td>CC</td>
<td>28</td>
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<tr>
<td><strong>TNFRSF1B</strong></td>
<td></td>
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<td>11695C&gt;T</td>
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<td>45</td>
<td>22</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>CT</td>
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<tr>
<td></td>
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<td>6</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>587T&gt;C</td>
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<td>35</td>
<td>30</td>
</tr>
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<td></td>
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<tr>
<td></td>
<td>CC</td>
<td>4</td>
<td>0</td>
<td>4</td>
</tr>
</tbody>
</table>

MAF-minor allele frequency; CMR-Clinical and morphological response; CR-Clinical response.
Patients received the initial dose of infliximab (5mg/kg/dose) in 0th, 2nd and 6th week. At the end of the first phase (after 10 weeks), the therapy effectiveness was determined.

Clinical criteria

After the initial period of therapy, its efficiency was evaluated by clinical criteria based on endoscopic examination and the CDAI change. Therapeutic response to infliximab was defined as CDAI below 150 or as a drop by more than 70 points, steroid withdrawal or healing of fistulas (Table 2). Patients were divided into two groups: group of primary responders (n=98) (within this group we distinguished patients with clinical and morphological response (n=51) and primary responders with clinical response but without morphological healing (n=47) and group of non-responders (n=18).

Genotyping

Patients’ samples were genotyped for SNPs on TNFRSF1A (T-610G, rs4149570; G-1488C, rs4149569) and TNFRSF1B (T11695C, rs976881; T587G, rs1061622) genes by PCR-RFLP.

Statistics

Results were evaluated by Fisher’s exact probability test and Odds ratio was used for statistical evaluation. P value below 0.05 was considered statistically significant.

RESULTS

Study population comprised 116 Caucasian patients treated with infliximab, overall 98 (84%) achieved response after the initial period of treatment (determined at 10th week after primary infliximab administration according to standard diagnostic criteria – CDAI and endoscopy examination), and 18 (16%) of patients had primary non-response. Genetic distribution of monitored SNPs and minor allele frequencies are shown in Table 3. Frequency of individual genotypes in all the monitored polymorphisms corresponded to Hardy-Weinberg equilibrium.

Frequencies of variant alleles of TNFRSF1A were comparable between responders and non-responders (38.8% and 36.1% for TNFRSF1A 4672G allele, 52.6% and 52.8% for 3794C allele, respectively). Variant allele TNFRSF1B 11695C was more common in non-responders (41.7% vs. 30.1%). Homozygotes for variant allele of TNFRSF1B 11695C were found more often (P=0.013; OR 5.89, CI 95% 1.6-22.1) in non-responders (n=5, 27.8%) than in responders (n=6, 6.1%). Homozygous patients for the variant allele TNFRSF1B 11695C were also more often resistant to the therapy (n=5; 27.8%), compared with patients with clinical and morphological reactions, where the frequency of this allele was 7.8 % (n=4; P=0.045; OR 4.52, CI 95% 1.06-19.29), and similarly when compared with a group of patients with clinical reaction (n=2; 4.3%, P=0.015; OR 8.65, CI 95% 1.5-49.9).

Correspondingly, the frequency of TNFRSF1B 587G allele in non-responders was 33.3% vs. 18.9% in responders. The frequency of this variant allele was lower in patients with clinical and morphological response 15.7% (P=0.031; OR 2.68, CI 95% 1.12-6.44 compared with non-responders).

DISCUSSION

In this work, we focused on polymorphisms located in a gene sequence for TNFR1 and TNFR2 (TNFRSF1A and TNFRSF1B). Infliximab effectiveness after the first 10 weeks of the therapy was studied in 116 patients. In 18 of them (16%), there was no therapeutic response (primary non-responders). Other patients (responders) reacted to infliximab by an improvement of clinical parameters of the disease (n=47; 41%), and 51 patients (43%) also revealed endoscopically verified morphological healing. An average age of patients included in the study was 38.2 years, and there were no significant differences between the group of responders and non-responders (38.5 and 36.4, respectively).

In the gene for TNFRSF1A, we monitored 2 SNP in the promoter area with an impact on the binding of transcription factors and expression of the receptor protein. The frequencies of individual variant alleles were comparable in both groups of patients (38.8% and 36.1% in responders and 52.6% and 52.8% in non-responders for -690T and -1488C, respectively). The frequencies of variant alleles correspond with results of another study, which was monitoring their occurrence in European population. In agreement with other authors, in our study, there was found no association between the presence of variant alleles and therapeutic effect of the infliximab therapy.

The frequency of the allele TNFRSF1B 11695A was in responders 30.1%. However, its frequency was significantly higher in non-responders (41.7%). The presence of homozygous genotype for this allele was associated with response failure (P=0.0132). Individuals homozygous for the variant allele totaled 27.8% in the groups of non-responders, compared with 6.1% in the group of responders. Since this polymorphism is located in the intron 1 sequence, we can hypothesize about the modification of posttranslational processes or binding to another polymorphism in the exon area. However, more studies are necessary to understand this mechanism. The distribution of variant allele 587G was 18.9% and 33.3% in responders and non-responders, respectively. Even though this difference in frequencies is considerable and corresponds with frequencies reported in other studies our results were not significant (P=0.073). Nevertheless, the frequency of this variant allele (15.7%) was lowest in patients with clinical reaction and at the same time morphological healing (P=0.031), compared with non-responders. The presence of variant allele leads to Met196Arg substitution in the fourth extracellular cysteine-rich domain. This substitution disrupts receptor functions from the perspective of its shedding activity. Changes in the protein primary sequence leads to a disruption of the ability to induce
NF-xB, and especially to the disruption of cross-talk between TNFR1 and TNFR2, where TNFR2 acts as an inhibitor of TNFR1-induced pro-inflammatory expression, and at the same time supports the pro-apoptotic signaling of TNFR1 (ref.10). Both these mechanism consequently modify the pro-inflammatory and pro-apoptotic balance towards the inflammatory one, therefore many authors associate the variant allele 587G with certain autoimmune diseases4,20 and lower effectiveness of the infliximab therapy9. Our results, however correspond with some authors13-15, are in contrast with others16-17. There is a need to carry out further studies of large groups of patients, which would precisely define possible genetic markers of the infliximab therapy effectiveness.

ACKNOWLEDGMENT

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CONFLICT OF INTEREST STATEMENT

Author’s conflict of interest disclosure: None declared.

REFERENCES

INTRODUCTION

Busulfan is an anti-cancer drug, in use since early 1960s. It is a cell cycle non-specific alkylating antineoplastic agent, in the class of alkyl sulfonates. Its chemical designation is:

1. 4-butanediol dimethanesulfonate (C₆H₁₄O₆S₂)

Fig. 1. Busulfan.

The drug is bifunctional alkylating agent, in which two labile methanesulfonate groups are attached to opposite ends of a four-carbon alkyl chain. In aqueous media, busulfan hydrolyzes to release the methanesulfonate groups. This produces reactive carbonium ions that can alkylate deoxyribonucleic acid (DNA), which is thought to be responsible for much of the cytotoxicity of busulfan. Thus; its mechanism of action through alkylation produces guanine-adenine intrastrand crosslinks. This occurs through a SN2 reaction in which the relatively nucleophilic guanine N7 attacks the carbon adjacent to the mesylate leaving group. This kind of damage cannot be repaired by cellular machinery and thus the cell undergoes apoptosis. Since 1970s busulfan high dose is used to replace total body
irradiation as myeloablative preparatory regimen before bone marrow/hematopoietic stem cell transplantation. Among the main challenges in this drug use is its wide inter- and intra-patient variability especially documented in oral busulfan pharmacokinetics. Use of the intravenous (I.V.) formulation might reduce this variability by eliminating variability in absorption, whereas variability due to drug metabolism remains. Furthermore, systemic exposure to intravenous busulfan appears to be relatively low in children compared with adults at certain doses; however, whether this should dictate a dose increase remains to be investigated in larger studies. Assessment of pharmacokinetic profiles may allow the characterisation of relationships between pharmacokinetic parameters and efficacy and toxicity. Therefore, further studies of pharmacokinetically guided busulfan administration may be needed to validate pharmacokinetic-pharmacodynamic relationships and to facilitate optimal dosage of the drug in practice.

In clinical practice the interindividual pharmacokinetics and dynamics variability necessitates AUC calculation based dose adjustment to achieve the target. Early pharmacodynamic studies suggested a significant relationship between high exposure and the occurrence of veno-occlusive disease (VOD) of the liver. However, pharmacodynamic studies are still required to define the relationship between busulfan exposure and optimal transplant outcome. For extensive pharmacokinetic study purpose, many samples were used, while some declare also in theory, only peak and trough levels should be necessary, but for assurance of reliability in clinical decision making, it must be possible to identify outlier values. Dupuis et al. for instance recommended that busulfan AUC be calculated for children using a four-sampling technique. In later publication it was reported that even fewer samples than four using 2 or 3 plasma busulfan concentrations can be used to reliably estimate busulfan AUC after I.V. administration in children undergoing HSCT. In the present three case studies including an adult and two paediatric patients, the unreliability of fixed dose and initial AUC estimates where busulfan intravenous busulfan has been prescribed as part of a myeloablative regimen prior to HSCT is the described.

CASE DESCRIPTION 1

A 49 year old Caucasian male with myelodysplastic syndrome had been scheduled for umbilical cord blood transfusion (HCT) using busulfan/fludarabine/thiotepa/ATG as conditioning regimen. Busulfan 0.8 mg/m2 I.V. 2 h lasting infusion at 6 hourly intervals was put in the protocol. Plasma samples were obtained immediately before infusion, immediately post infusion, at 4, and 6 h from the start of initial dose infusion. The high performance liquid chromatography (HPLC) system consisted of an isocratic pump with a wavelength detector, using an automatic sampling system (ECOM, Prague, Czech Republic) was applied to determine plasma busulfan concentrations. The ultraviolet detection was carried out at 256 nm and the injected volume was 20 μL and the chromatograms were processed by Clarity software (Data Apex) with the retention time 5.8 min for busulfan and 11.8 min for internal standard, respectively. The calibration curve was evaluated as linear relationship between the peak area ratio for busulfan and internal standard using the calibration prepared from pooled human serum spiked with stock solution of busulfan as explained elsewhere. Busulfan plasma concentration as measured by the validated method revealed undetectable in trough or steady state (Css) sample, 194.3 μg/L, 53.9 μg/L, undetectable in samples immediately post two hour lasting infusion (C2), at 4 h (C4), and 6 h (C6) from the start of infusion, respectively. The AUC drawn from these concentrations was unacceptably very low bearing only 496.4 μg/L.h. Despite continuation with the initial dosage scheme, samples immediately before the 5th infusion, immediately after the end of infusion, 4 h after the start of 5th infusion, and at 6 h revealed concentrations 556.4 μg/L, 1604.9 μg/L, 711.7 μg/L, and 430 μg/L respectively (Fig. 2). The resulting AUC of approximately 5620 μg/L.h as calculated using the trapezoidal rule has been accepted for being within the required myeloablative target.

**Fig. 2.** (Case 1) demonstrating significant busulfan blood concentration versus time profile difference in the same subject with the same I.V. dosing regimen in an adult patient.
CASE DESCRIPTION 2

The 2 years old girl has been admitted with diagnosis of Langerhans cell histiocytosis and secondary haemophagocytic lymphohistiocytosis. After the failure of conventional chemotherapy, the patient has been included in the schedule for HSCT to be proceeded by myeloablative preparative regimen, which contained high dose busulfan and fludarabine including prophylaxis against graft versus host disease (GVHD) with alemtuzumab (Campath) administration. The patient started I.V. busulfan on actual body weight basis, i.e., 2 mg/kg (17 mg/dose at 12 h intervals with infusion rate of four hours). Blood samples for therapeutic monitoring were collected starting with trough (before the 4th infusion), then immediately after the end of the 4th infusion, 8 h, and 12 h from the start time of the 4th dose. Surprisingly, very low (near the lower detection limit) steady state concentration (trough) consequently leading to unacceptably low exposure expressed by low AUC (1284 μg/L.h) was revealed. After this evidence the dosing interval was shortened to 6 hourly regimen with the rate of infusion also switched to 2 h (1.2 mg/kg/dose i.e., 10.5 mg at 6 hourly intervals (7 doses). Later, the AUC of 6139 μg/L.h, which is within the required target, has been achieved. In addition to change of dosing interval, there was a dose escalation by one extra dose than previously planed. Due extremely low initial busulfan bioavailability, blood sample has been preserved for pharmacogenetic analyses or eventual rapid metabolism based on enzyme polymorphism. Further analysis for genetic polymorphism in this case did not prove genotype, which may explain the underlying reason for faster elimination of the drug given by standard dose (Fig. 3). Allogenic HSCT using umbilical cord blood with 9/10 HLA (human leukocyte antigen) matched, i.e., with Cw mismatch 4.4x10/5/ kg CD34+ (cluster of differentiation) cell count has been conducted. Thereafter, the patient was in excellent clinical state, mostly nonfebrile, in exception of elevation of c-reactive protein (CRP) attributed to the underlying disease activity still persistent shortly after HSCT. On further follow-up also the patient was in good condition, except for mild sign of mucositis. Since D+1 (1st day after HSCT), Defibrotide 25mg/kg as prophylaxis against VOD has been administered. For unexplained CRP elevation, corticosteroid therapy (1 mg/kg) has been applied. CsA blood level was also low after the first three days of administration and the dose has been escalated to reach the recommended target. Since D+8 fever episodes dictated combination antibiotic therapy (Amikacin + Targocid + Meronem). On D+11(11th day after HSCT), Defibrotide has been terminated for absence of VOD symptoms. Corticosteroid therapy, which was terminated on D+15 (15th day after HSCT), has been restarted at a dose of 1 mg/kg/day due to fever up to 39 °C and elevation of inflammatory markers. The effect of cortico-therapy was evident after initial dose leading to improvements of clinical state and laboratory markers. Engraftment with leukocytes was evident by D+22 (22nd day after HSCT). Later during post transplantation period, hemoculture revealed Candida lusitaniae, which has been successfully treated with combination of posaconazole and mycamine. Later follow-up revealed post transplantation lymphoproliferative disorder (Epstein Barr Virus (EBV)-induced diffuse large B-cell lymphoma (DLBCL), for which the patient has been successfully treated by totally 8 doses of rituximab, and now is on regular follow-up management.

Fig. 3. (Case 2) demonstrating significant busulfan blood concentration at trough, 2-12 h from the start of infusion (C2-C12) profile difference in the same subject with the same total I.V. dose, but at different dosing interval (12 hourly red line versus 6 hourly blue line) in a paediatric female patient illustrating considerable variability.
CASE DESCRIPTION 3

A 2 year old male infant with acute myeloid leukaemia was scheduled for busulfan myeloablative therapy (1.2 mg/kg based on his actual body weight of 11.1 kg). For planed Allogenic HSCT, from sibling (sister) donor, Busulfan + Cyclophosphamide + Melphalan (Bu+Cy+Me) pre transplantation conditioning regimen including cyclosporine-A as prophylaxis against graft versus host disease (GVHD) targeting trough concentration of 100-150 μg/L has been indicated. Busulfan estimated dose of 13 mg has been administered I.V. over 2 hour infusion at 6 hour intervals. Blood samples for therapeutic drug monitoring (TDM) purpose have been obtained starting from trough after 4th dose, at peak (immediately after the completion of the 5th infusion), at 4 h, and 6 h counted from starting time of the 5th infusion. These four samples were analysed by HPLC method as explained above and used to calculate AUC at steady state based on our already developed limited sampling strategy for TDM of busulfan. AUC C_{trough-6} calculated according to the trapezoidal rule revealed potential overexposure (11135 μg/L.h) as also illustrated on Fig. 3. Based on this observation the initial dose has been reduced by 2 mg per dose. However, concentrations after the dose adjustment also showed cumulative overexposure, despite evident dose reduction. Finally, we decided to further reduce the dose to only 8 mg per dose. In this case, after more (approximately 40 %) dose reduction, the AUC still proved to remain above the target required on the protocol. As far as outcomes is concerned, since 12th day post transplantation, gradual manifestation of hepatopathy with increased bilirubine-mia – max. 70 μmol/L, and liver aminotransaminases (GMT max. 11, ALP max. 10, AST 3, ALT 5 μkat/L), all of which restored within 10 days without further therapeutic interventions have been observed. For suspected veno-occlusive disease (VOD), defibrotide has been administered only for several days, and discontinued for not fulfilling the VOD criteria (symptoms). To date (28 days post transplantation) the child is without signs of liver impairment, with complete engraftment (of both leucocyte and thrombocyte elements). The patient suffered an episode of haemorrhagic cystitis, most probably of BK virus (a member of polyomavirus) aetiology, otherwise is doing well and still in institutional care at the transplantation unit.

DISCUSSION

High-dose busulfan is widely used instead of total body irradiation (TBI) before bone marrow or haemopoietic stem cell transplantation (HSCT) conditioning regimen in both adults and children. Its considerable pharmacokinetic variability and worrying adverse effects in case of extreme exposure or therapy failure in case of under-exposure warrant pharmacokinetic monitoring in both oral and intravenous forms. Previous works suggested that test pharmacokinetic study enables dose prediction for all 16 doses given every 6 h. However, extensive case series observations and further studies indicate that the drug shows intra-individual variability challenging the first dose analysis based prediction. Variation in the area under the concentration/time curve (AUC) of busulfan could results in substantial risk of over or under treatment with excess risk of toxicity or relapse. Thus intensive therapeutic drug monitoring primarily by determination of drug plasma levels using validated methods such as high performance liquid chromatography (HPLC) as explained elsewhere and followed by AUC calculation to be within required targets maybe helpful. In conventional sampling regimen for determining AUC after oral administration, over 10 samples were used to assure accurate tracking of erratic absorption. In limited sampling strategy theoretically, only peak and trough levels may be necessary, but for assurance of reliability in clinical decision making with possibilities to identify outlier values, at least 4 samples may be required. Based on AUC observed, available guidelines recommend dose escalation or reduction up to 20 % of the initial dose or according to a conventional formula to obtain target AUC between 5000 and 7000 μg/L.h myeloablative range. However, in the demonstrated case of our adult patient (Case 1) such escalation of doses might lead to potentially toxic exposure. Provided the same approach of clinical
and laboratory procedures applied, significant differences in concentrations post initial and later doses may be only explained by intra-individual variability in this adult patient as we previously confirmed in paediatric patients both on oral and intravenous forms of the drug\textsuperscript{9}. The case published just two years ago by Johnson-Davis et al.\textsuperscript{11} also demonstrated significant intra-patient variability especially using concomitant drugs warranting more concern to further monitor the therapy. McPherson et al.\textsuperscript{12} reported that hepatic veno-occlusive disease occurred in 32\% out of 25 patients, but not evidently associated with busulfan AUC. However, low AUC was associated with partial donor chimerism, while full donor chimerism has been observed in cases within target AUC. Nevertheless, there are other reports suggesting that high plasma concentrations of busulfan have been linked to the occurrence of hepatic veno-occlusive disease, a severe complication associated with a high mortality\textsuperscript{13}. There is an evidence that busulfan pharmacokinetics showed high inter- and intra-individual variability in HSCT using a targeted busulfan/fludarabine regimen, which indicates the need for intensive monitoring and dose adjustment to improve the outcome of HSCT (ref.\textsuperscript{14}). Therapeutic monitoring of intravenous busulfan is to increase the efficacy and safety of busulfan-based conditioning protocols in paediatric HSCT recipients\textsuperscript{15}. Significant intra-individual variability exists in the apparent oral clearance of busulfan and follow-up therapeutic drug monitoring is recommended particularly if the desired target AUC is narrow\textsuperscript{16}. Interestingly, the paper published by Bartelink et al.\textsuperscript{17} expressed that dose adjustment based on AUC of day 1 led to unexpectedly low AUC with unfavourable consequences as demonstrated clearly at least in two of their patients. That is why any changes made based on early monitoring should be followed by another control of blood levels to know actual AUC for eventual dose readjustment as we have published in our preliminary report of limited sample based therapeutic drug monitoring of busulfan in children\textsuperscript{18}. In contrast to the adult (Case 1), in a paediatric patient (Case 2), to our surprise the AUC later showed unacceptably high and led us to significant dose reduction (approx. by 40\% from the initial one). Actual body weight based busulfan dosing schedule (Table 1.) counts with relatively higher dose requirements for younger children and infants with smaller body weight; however, in our case 1, the dose has been finally reduced to 8 mg/dose corresponding to the dose recommended for subjects of actual body weight greater than 34 kg due to AUC evidently exceeding the required target AUC between 5000 and 7000 μg/L·h.

It is worsewise to remind that dosing in adults is similarly based on actual body weight, but at relatively lower doses per kg weight than in children and infants are allowed. Nevertheless the extended interval dosing in our female patient also showed significantly different AUC on the same bodyweight based dose for yet not have been well explained reason, meanwhile faster metabolism at initial dose cannot be excluded in this case. According to our observations the relevance of bodyweight based fixed dose busulfan dosing scheme requires review or otherwise warrants the use of validated limited sampling strategy, which may make possible meaningful and more accurate use of busulfan for pre HSCT preparative regimen\textsuperscript{19}.

**CONCLUSIONS**

1) AUC after initial doses of busulfan may not predict exposure throughout therapy, due to significant intra-patient variability conditioned by several patient factors.

2) Follow-up therapeutic drug monitoring has been shown to be highly required tool to guarantee aimed target in both adult and children patients with careful interpretation of drug levels considering all influential factors.

3) Body weight based fixed dose busulfan dosing needs review as shortcomings exist and individual approach is still of vital importance. As overall conclusion, we recommend therapeutic drug monitoring after day 1 of application and follow-up between next doses instead of predicting the pharmacokinetics for the whole 16 doses based only on first test dose to secure that the right dose is provided in this challenging intervention due to considerable intra-individual variability.

**ACKNOWLEDGEMENTS**

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**REFERENCES**


**Table 1. Conventional actual body weight based busulfan dosing scheme in children.**

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<tr>
<th>Actual body weight (kg)</th>
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STŘEDA 11.9.

14:00  zahájení 63. Farmakologických dnů

14:15–15:00
Zahajovací přednáška
Vybrané aspekty vývoje nových léčiv
Král V (VŠCHT a Zentiva, Praha)

Sekce experimentální farmakologie a toxikologie:

15:00  Elucidation of the transformation of nabumetone to the active metabolite, 6-methoxy-2-naphthylacetic acid (6-MNA)

15:20  Zlepšenie štandardnej anti-artritickej liečby pridaním N-feruloyl serotoninu k metotrexátu hodnotené na modeli adjuvantnej artritidy
Kuncírová V, Ponišť S, Draší F, Mihalová D, Dobiašová M, Harmatha J, Nosál R, Bauerová K

15:40  Ethanol a jeho hlavní metabolit acetaldehyd ovlivňují inward rectifier draslíkový proud IK1 u komorových srdečních buněk potkanů
Bebarová M, Matejovič P, Pásek M, Šimurdová M, Šimurda J

16:00  Polyfenolické zmesy a experimentálné vyvolaná alergická astma
Kazimierova I, Jošková M, Pecháňová O, Šutovská M, Fraňová S

Přestávka: 16:20–16:40

16:40  BKCa kanály a experimentálna allergická astma
Kocmálová M, Oravec M, Adamkov M, Šutovská M, Fraňová S

17:00  Vplyv roflumilastu na in vivo a in vitro reaktivitu dýchacích ciev a apoptózu u ovulácinom sensibilizovaných morciat
Medvedová I, Pršo M, Eichlerová A, Mokrý J, Mokrá D, Mikolka P

17:20  Predklinické štúdie nového derivátu kvercetinu-inhibitora aldoketoreduktáz AKR1B1 a AKR1B10. Význam v modeloch chronických diabetických komplikácií, v modeli zápalu a rakoviny
Miláčková I, Šoltésová-Prnová M, Majecková M, Sotníková R, Navarová J, Račková L, Díez-Dacal B, Perež-Sala D, Enayat S, Dobrovská A

17:40  Štúdium molekulových foriem cholínesteráz v srdci
Dingová D, Krejčí E, Hrabovská A

18:00  On the molecular pharmacology of oxidative burst inhibition in human neutrophils
Nosál R, Dražíková K, Jančinová V, Perečko T, Harmatha J, Šímikdal J

19:00  Společenský večer

ČTVRTEK 12. 9.

Sekce experimentální farmakologie a toxikologie – pokračování

9:00  Iron-chelating agents and acute myocardial infarction: in vitro and in vivo study
Filipský T, Říha M, Hrdina R, Mladěnka P

9:20  Study of Simvastatin & Creatine citrate therapy in animal model of dementia by in vivo proton and phosphorus magnetic resonance spectroscopy
Kašparová S, Bačík L, Tušková R, Kebs A, Dubovický M

9:40  Developmental manipulation of monoaminergic systems affects neurobehavioral and neuroendocrine regulations
Dubovický M, Császárová E, Ujházy E, Sediáčková N, Mach M
10:00 Úloha nikotínových receptorov vo fyziolózii srdca  
Mrvová K, Kučera M, Hrabovská A

10:30–11:30 Shromáždění členů ČSEKFT

12:00–14:00 Oběd, Diskuse u posterů

**Klinická sekce:**

14:00 Farmakoterapie septických nezralých novorozenců v kritickém stavu. Význam komorbidit. 1. gentamicin při současném perzistujícím ductus arteriosus.  
Martinková J, Pokorná P

14:20 Farmakoterapie septických nezralých novorozenců v kritickém stavu. Význam komorbidit. 2. gentamicin při současné perinatální asfyxii.  
Pokorná P, Martinková J

14:40 Analysis of non-steroidal anti-inflammatory drug usage and risk perception in hospitalized patients  
Varga Z, Kristová V, Kriška M

15:00 The specific issues in pharmacotherapy in elderly patients – misprescription and underprescription  
Wawruch M, Slezáková V, Murin J, Dukat A, Bozik M, Potocarová M

15:20 Issues of oral anticoagulants prescription in elderly patients with atrial fibrillation  
Slezáková V, Potocarová M, Murin J, Dukat A, Bozik M, Petrová M, Wawruch M

15:40–16:00 přestávka

16:00 Vieme predchádzať nežiadúcim interakciám v praxi.  
Kriška M

16:20 Effect of methycobalamin application in patients with autism  
Čorejová A, Rauová D, Jánosičková D, Pospíšilová V, Miková M, Repík J, Lakatošová S, Hrabovská A, Kyselovič J

16:40 Current challenges of body weight based intravenous busulfan dosing versus dose adjustment based on therapeutic drug monitoring.  
Tesfaye H, Branova R, Riha J, Sdlaček P, Vydra J

17:00 Impact of diabetic state in patients with end-stage heart failure and its association with cardiac expression of microRNAs.  
Dóka G, Křenek P, Klimas J, Goncalvesová E, Kyselovič J

17:20 Differential gene expression of important factors in human epicardial adipose tissue and left ventricular myocardium in end-stage heart failure.  
Mlynárová J, Dóka G, Musil P, Hulman M, Goncalvesová E, Kyselovič J

17:40 Effect of fenugreek seeds enriched diet on endothelial dysfunction in mild diabetes and its relation to nitric oxide and epoxyeicosatrienoic acids pathway  

18:00 First clinical pharmacology department in the world  
Strojil J, Jezdinský J

18:30 Volný večer

**PÁTEK 13. 9.**

**Sekce molekulární a buněčné farmakologie:**

9:00 Polymorphisms in protein C gene promoter region and endothelial protein C receptor gene as predisposing factors for venous thrombosis  
Horáková K, Bartošová L, Kolorz M, Hanzlíková P, Wróblová K

9:20 Association of haplotypes HLA-DQ2, HLA-DQ8 and polymorphism G-308A in TNF-alpha gene with coeliac disease  
Wróblová K, Kolorz M, Horáková Z, Pav I, Bartošová L

9:40 TNFRSF1A and TNFRSF1B gene polymorphisms and their impact on effectivness of therapy with infliximab  
Kolorz M, Wroblów K, Baťovský M, Zbořil V, Mokřanová J, Bartošová L
10:00 ACTH-mediated regulation of the human MC2R.
Kiliánová Z, Basora N, Payet M, Gallo-Payet N

10:20–10:40 přestávka

10:40 Diagnostika rezistentných form tuberkulózy – konvenčné metódy versus molekulárnogenetické metódy
Porvazník I, Mokrý J, Solovič I

11:00 Aryl hydrocarbon receptor and its crosstalk with glucocorticoid receptor in human placental barrier
Stejskalová L, Rulcová A, Vrzal R, Dvořák Z, Pavel P

11:20 Flavonoids from Morus alba affect cell cycle of human cancer cells and inflammatory response
in macrophage-like cells.

11:40 Commonly measured biomarkers of oxidative stress do not reliably predict cardiovascular impairment
Mladěnka P, Filipsky T, Vávrová J, Holečková M, Palička V, Hrdina R

Varia:

12:00 Application of computer-based modeling in the analysis of cardiovascular regulation mechanisms
Vojtko R, Petrová M, Villaris R, Kristová V

12:20 The Protective Effect of Pyrimidine Nucleosides and their Combinations on HaCaT Keratinocytes Treated
with 5-FU: MTT, NTCA and RTCA Tests
Hartinger J, Veselý P, Matoušková E, Petruželka L, Netíková I

12:40 Závěrečné slovo a ukončení 63. Farmakologických dnů
INSTRUCTIONS TO AUTHORS

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