Prague Medical REPORT

(Sborník lékařský)

Multidisciplinary Biomedical Journal
of the First Faculty of Medicine,
Charles University in Prague

Vol. 109 (2008) Supplement

Dear Colleagues,
Dear Readers,

The Supplement of Prague Medical Report, Vol. 109 (2008) contains contributions to be presented at the 58th Czech and Slovak Pharmacological Meeting (Pharmacological Days), which will take place in Prague on September 3–5, 2008.

The First Faculty of Medicine, Charles University in Prague is pleased to host this event, representing a traditional gathering of Czech and Slovak pharmacologists and scientists interested in related fields of biomedical sciences, in the year of the 660th anniversary of its existence.

We would also like to cordially welcome guest speakers from Germany, Norway, UK and USA.

The organisers hope that you will enjoy both the scientific and cultural aspects of this Meeting.

Professor Tomáš Zima, MD., DSc.
Dean of the First Faculty of Medicine,
Charles University in Prague

58th Pharmacological Days

September 3–5, 2008 in Prague

Collection of Abstracts

ČESKÁ SPOLEČNOST
PRO EXPERIMENTÁLNÍ
A KLINICKOU FARMAKOLOGII
A TOXIKOLOGII ČLS JEP

Czech and Slovak Pharmacological Meeting (58th Pharmacological Days)

Prague, September 3–5, 2008

The content and language editing is under the responsibility of authors.

Prague Medical Report (Prague Med Rep) is indexed and abstracted by Index-medicus, MEDLINE and PubMed.

Why So Many Cytochromes P450

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Key words: Cytochrome P450 – Flexibility – Active site

This project was supported by the grant number GA ČR 305/08/0535 and MSM ČR No. 6198959216.

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Introduction Cytochromes P450 are ubiquitous in the nature. Although the mechanism of their enzyme action is in the majority of reactions almost the same (in this respect the NO synthase ia also a P450 enzyme), their number is about 60 in animals and more than several hundreds in the plants. The reason for their multiplicity is evidently based on the properties of their active sites.

Methods Active sites of P450 enzymes were studied both by experimental as well as theoretical methods. Absorption spectroscopy at high pressure and resonance Raman spectroscopy were the experimental methods, whereas methods of molecular dynamics were chosen to evaluate the temperature B factors and radii of gyration.

Results and Conclusions Results show that cytochromes P450 differ in their ability to accommodate the substrates not only because the amino acid composition of the active site, but also because of the flexibility of their structure. This property is at least of the same importance for successful enzyme catalysis as the proper amino acid residue in the active site.

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Determination of Transport Kinetics of Somatostatin Receptor-specific Peptide to Opossum Kidney Cells

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Key words: Kidney transport – Ligand Tracer – OK cells – Radiolabelled peptides

This study was supported by grant GA ČR No. 305/07/0535.

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Introduction Radiolabelled somatostatin analogues represent very promising agents for visualisation and treatment of endocrine tumours. As high and long-term renal radioactivity retention and thus the radiation dose delivered to the kidney is a major dose-limiting factor in peptide receptor radionuclide therapy, analysis of renal transport of such peptides forms is an important part of their preclinical investigation.

Present study was aimed at the comparison of two methods, which enable the determination of internalization of radiolabelled peptides to selected cell line *in vitro*. In the study the result of a classical method and the recently introduced new automated analysis of an uptake and internalization with an employment of the instrument Ligand Tracer Yellow (Ridgeview Instruments AB, Uppsala, Sweden) were confronted.

Methods Radiolabelled somatostatin derivative [^{111}In-DOTA]-1-NAL^3-octreotide (^{111}In-DOTA-NOC) was used in this study. For *in vitro* uptake experiments opossum kidney cells (OK cells) were used. The reason of their utilization lies in the fact that the multiligand, endocytic receptors megalin and cubilin are responsible for the uptake of radiolabelled peptides in renal proximal tubules, and OK cells retain characteristics of proximal tubular epithelial cells.

The experiments were performed either by classical incubation technique or with an employment of a semi-automated rotating cell dish with *in situ* reference area.

The first method was made on Petri dish. Renal OK cells were grown in plastic 75 cm^2 culture flasks in MEM supplemented with 2 mM L-glutamin, 1% NEAA and 10% FCS in 5% CO_2 atmosphere at 37°C. For experiments, OK cells were grown to confluency on plastic Petri dishes (6 cm diameter). Confluent monolayers were washed (2×) first with PBS and incubated simultaneously for indicated intervals (0, 15, 60, 120 and 180 minutes) with the same concentration 1 nM of ^{111}In-DOTA-NOC in Ringer solution (37°C). At the end of the incubation, the uptake buffer was

discarded and the dishes with cells monolayers were rapidly rinsed with ice-cold PBS (6×). The cells were disintegrated by Triton X-100 (0.1% v/v) in 10 mM MOPS. To elicit the measuring error caused by nonspecific sorption to the cells and Petri dishes, the radioactivity uptake at time 0 was used as a blank value where the internalization medium was discarded immediately after addition. The intracellular radioactivity was normalized to the cell protein content by the BCA method.

Measurement of In^{111} activity was performed by a gamma spectrometer 1480 Wizard^{TM} 3$^{//}$ (Wallac, Finland). Radioactivity of all measured samples was compared with those of standard samples.

Ligand Tracer Yellow was used for the automatic real-time cell internalized radioactivity detection. Only one Petri dish was needed for the analysis. Cells were seeded in mass 5×10^5/2 ml of medium in tilted dish to small maximally 2 cm long region. After their attachment 2 ml of added medium was removed and up to 10 ml of fresh medium was added. Cells were incubated one day. The experiment using Ligand Tracer Yellow was performed with removed culture medium and added up to 2 ml of Ringer solution with 1 nM ^{111}In-DOTA-NOC. A scintillation detector measured increasing radioactivity from internalized peptide in cells using like a reference area opposite part of dish without cells. An analysis was run maximally for three hours. When the analysis was over, cells were disintegrated, and radioactivity content and cell protein mass were measured as by the first method.

Results and Conclusions The results obtained by manual method showed that ^{111}In-DOTA-NOC entered cells quite easily, and its internalized amount increased during the incubation time. But the fastest movement of the peptide through cell membrane was during the first hour of incubation. Then the velocity of the peptide passing through a cell membrane slowed down with increasing incubation time and the radioactivity valuation at 120 and 180 minutes was not significantly different from the worth of radioactivity at 60 minutes.

The real-time detection of ^{111}In-DOTA-NOC internalization by OK cells on Ligand Tracer Yellow instrument was also performed for three hours incubation. The great advantage of this method was simple using of only one Petri dish for the three hours non-stop detection. The result of this measurement was a lot of points, which increased its valuation of radioactivity concentration with levelled up time. Similar results were detected in the later procedure in comparison with the manual method. It means, the uptake of radiolabelled peptide was very rapid during first hour and after first sixty minutes it slowed down and was nearly invariable.

The results of both methods were comparable. The both have showed good uptake of ^{111}In-DOTA-NOC to OK cells, which is due to its lipofility character. They have demonstrated the critical time for peptide internalization, which is up to first sixty minutes.

Obtained results showed that both methods provide valuable information concerning specific peptide internalization by cells. The advantage of Ligand Tracer Yellow analysis is the more convenient way for radiolabelled peptide internalization analysis. It allows real-time, clean, less challenging, low price and faster assays. On the other hand, there are also some limitations of this procedure. The first one is connected with a difficult control of constant temperature during the experiment. The heat system installed in the apparatus contributes to the fast solution evaporation, which enables sticking of reaction solution on the cells. The false positive results could be thus obtained. This process can be avoided placing the machine in a thermoregulator.

The other disadvantage is that the Ligand Tracer Yellow instrument doesn't allow a determination of radioactivity distribution between cells and medium. In addition to it, significantly higher specific activity should be used in the rotating cell dish method in comparison with the classic manual measurements.

When we look apart from the above mentioned limitations, we can conclude that the use of Ligand Tracer is a good choice for peptide internalization analysis.

Effect of Chalcones in the Conditions of Kidney Ischemia-reperfusion (a Pilot Study)

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Key words: Chalcones – Kidney ischemia-reperfusion – Antioxidants

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Introduction Chalcones are substances with antioxidative effect *in vitro* in comparison with butylhydroxytoluene (BHT). *In vitro* testing confirmed their antibacterial activity against Staphylococcus aureus too.

Aim of the study The aim of the study was to monitor antioxidative effect of two chalcones (trihydroxychalcone and trihydroxydihydrochalcone) in the conditions of of kidney ischemia-reperfusion in experiment *in vivo*.

Material and Methods The study was performed on Wistar SPF male. After 10-day acclimatization, the animals were split up into four groups on a random selection basis. Both two chalcones in the doses of 10 mg/kg were administered to the treated groups in 0.5% Avicel solution once a day. To the placebo group, only 0.5% Avicel solution in the dosage of 2 ml was administered, again orally once a day. The last group was intact.

On completion of the medication, laparotomy was performed in experimental animals in general anesthesia, left renal artery was prepared and isolated and kidney ischemia was induced using "bulldog" vascular clip for 60 minutes. Afterwards, the clip was released and kidney reperfusion followed for 10 minutes. On completion of the reperfusion, the animals were exsanguined and malondialdehyde (MDA) level in serum using TBARs method, as well as superoxide dismutase (SOD), glutathione peroxidase (GSHPx) and overall antioxidative capacity (AOC) were determined. Also, kidney tissue samples were taken for the purposes of histopathology examination. The acquired values of monitored laboratory parameters were statistically evaluated using ANOVA test.

Results Statistically significant difference in SOD, GSHPx and MDA values ($p \leq 0.01$) was identified in the group treated with both two chalcones in comparison with the control group. In statistical comparison, the acquired values of overall antioxidative capacity showed insignificant changes.

Histopathology examination results:

Treated groups: The affection in the treated groups is evaluated as light, only sporadically up to moderate.

Placebo group: The affection in the placebo group is predominantly diffuse, of moderate to severe extent.

Discussion Statistically significantly higher levels of both enzymes, detected in the treated groups, indicate the preparedness for liquidation of superoxides, elimination of hydrogen peroxide and other free radicals causing kidney tissue damage after reperfusion. We assume that this is the result of the prior preventative supplementation of animals in these groups by the substances with proven antioxidative effect *in vitro*. In comparison with the placebo group values, a light increase in AOC values, presented extracellularly, was observed in the treated groups. The results of the statistical comparison of secondary toxic product of lipoperoxidation MDA values between the treated and placebo groups show significant changes too.

Conclusion The results of biochemical examination show antioxidative effect of both two chalcones. The results of histopathological examination correlate with them partially only.

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Effect of Stereoisomery on Antiarrhythmic Impact of Newly Synthesized Compound 44Bu

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Key words: Cardiotoxicity – Aconitine – Rat – Stereoisomery – Aryloxyaminopropanols

This project was supported by grants GA ČR No. 305/06/0863 and IGA MZ ČR No. NR9126-3/2006.

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Introduction Aconitine – pentacyclic diterpene alkaloid from the plants genus Aconitum (Aconite) is one of the most violent natural (native) toxins. It is a neurotoxin, which blocks inactivation of voltage activated sodium channels in excitable tissues and causes their persistent opening. Aconitine therefore permanently depolarises membranes, which results in clinical manifestations, e.g. supraventricular and ventricular arrhythmias. Treatment of the aconitine-induced ventricular arrhythmias is just supportive in clinical practice because no specific antidote has been found yet.

Previous experimental results confirmed that 44Bu is more efficient in suppressing the aconitine-induced ventricular arrhythmias than standard antiarrhythmic drugs such as lidocaine and propaphenone.

The aim of this work was to evaluate the effect of the racemate, R and S stereoisomers of the 44Bu compound on cardiotoxicity of aconitine in prophylactic administration.

Methods The newly developed compound (draft name 44Bu) is an original compound that was synthesized by the staff at our Faculty of Pharmacy. No pharmaceutical company has taken part in its development. The 44Bu is optically active. Due to the chirality there exist two molecules – enantiomers: R-isomer and S-isomer. Racemate contains the same number of molecules of the R and S enantiomer, therefore it is optically inactive. Individual enantiomers can have

various pharmacological or toxic characteristics, which is given by their different interactions with biomolecules of an organism (receptors, ion channels...).

Experiment was performed in vivo on 41 male Wistar laboratory rats (220±81 g). Animals were divided into four groups: Rac (n=10), R (n=7), S (n=8) and K (control) (n=16).

1. The animals were anaesthetized by i.m. administration of a mixture of 1% ketamine and 2% xylazine in a dosage of 0.5 ml/100 g.
2. The tested substances of 44Bu (racemate, R and S isomer) resp. saline solution were administered as prophylactic agents i.v. into the exposed vena jugularis. After 2 minutes aconitine was administered into the vena jugularis, also.
3. Animals were continuously monitored on the Seiva Praktik ECG machine for 25 min after the administration of 44Bu substances.

Dosage of aconitine: 30 μg/ kg of animal weight (~0.046 μmol/kg).

Dosage of tested compounds: 1.5 mg/kg of animal weight (~3.72 μmol/kg).

There were monitored 6 types of atrial and ventricular arrhythmias (see below) and blockade of conduction (the 1st degree blockade of conduction and the 2nd degree atrio-ventricular block without any close specification of the blockade type).

Atrial arrhythmias: supraventricular premature beats; atrial fibrillation.

Ventricular arrhythmias: ventricular premature beats – discrete or in salvos; ventricular premature beats – bigeminies and trigeminies; ventricular tachycardia and ventricular fibrillation.

In monitored types of arrhythmias we evaluated occurrence percentage in each monitored group and time of the first occurrence. There was also evaluated the survival rate of animals during the 25 minute-long experiment (mortality), and the effectiveness of tested substances on individual arrhythmia types (Profile of arrhythmias in each group = ratio of individual arrhythmia types on the overall composition of arrhythmias in the group).

The programme Unistat 5.1 was used to carry out statistical analyses. We used χ^2-test of two variables to compare the occurrence frequency of selected arrhythmia types in tested groups of animals and for the mortality assessment. Changes of time of the occurrence of these arrhythmias were assessed using the nonparametric Mann-Whitney test.

Results and Conclusions

- S-isomer and racemate significantly ($P<0.01$) decrease the occurrence of dangerous types of arrhythmias, such as ventricular tachycardia and ventricular fibrillation against control.
- Racemate is better than S-isomer but nonsignificantly. Unfortunately it seems, that racemate induces AV blockades.
- R-isomer proved the pure antiarrhythmic effect, but differences between isomers were nonsignificant.

Table 1 – The time onset (minute) and occurrence (%) of the monitored type of arrhythmia. Profile of arrhythmias (%) and total number of monitored arrhythmias per one animal in each group

	Group K, n=16		Group R, n=7	
Type of arrhythmia	The time onset (min.)	Occurence (%)	The time onset (min.)	Occurence (%)
SVPB	0.63	69	3.57*	29
AF	1.27	50	0.35	14
VPB-DS	0.87	94	7.15**	71
VPB-BT	1.03	63	8.65**	86
VT	1.70	100	6.18*	57**
VF	3.90	94	9.93*	29**
Blockade	1.13	75	5.85*	29*°°
Mortality	5.10	100	11.50	29**
	Group S, n=8		Group Rac, n=10	
Type of arrhythmia	The time onset (min.)	Occurence (%)	The time onset (min.)	Occurence (%)
SVPB	12.80*	25*	14.33**	60
AF	1.90	13	13.00	20
VPB-DS	5.48*	25**	17.83**	60*
VPB-BT	7.23*	63	16.33**	90
VT	10.08**	50**	23.00*	20**
VF	23.00$^{n.ev.}$	13**	No occur	0**
Blockade	14.20	25**°°	15.62**	90°°
Mortality	No occur	0**	No occur	0**
Ratio of arrhythmia type on the overall composition of arrhythmias in each group (%)				
Type of arrhythmia	Group K n=16 Ratio (%)	Group R n=7 Ratio (%)	Group S n=8 Ratio (%)	Group Rac n=10 Ratio (%)
SVPB	13	9	12	18
AF	9	5	6	6
VPB-DS	17	23	12	18
VPB-BT	11	27	28	26
VT	19	18	24	6
VF	17	9	6	0
Blockade	14	9	12	28
Total per one animal	5.4	3.1	2.1	3.4

SVPB = supraventricular premature beats; AF = atrial fibrillation; VPB-DS = ventricular premature beats – discrete or in salvos; VPB-BT = ventricular premature beats – bigeminies and trigeminies; VT = ventricular tachycardia; VF = ventricular fibrillation; BL = blockade of conduction

$^{**}P < 0.01$; $^{*}P < 0.05$ – significancy of all group vs. control group K

$^{°°}P < 0.01$; $^{°}P < 0.05$ – significancy of stereoisomers (groups R, S) vs. racemate (group Rac)

n.ev. = not evaluated (occurence VF only in one animal);

First part of table: 100% = total number of experimental animals in the given group (n)

Second part of table: 100% = total number of monitored arrhythmias in the given group (n)

- All forms of the 44Bu have an ability to delay the start of arrhythmias. The best postponing effect have racemate and then S-isomer. R-isomer had the smallest impact.
- After the prophylactic administration of the tested substances the profile (composition) of individual arrhythmia types changed, which is very beneficial especially when the occurrence of the most dangerous arrhythmias decreases.
- Racemate and S-isomer have the best decrease on the mortality reduction of aconitine intoxicated animals.

Comparison of the Effects of Activated Neutrophils with the Action of Reactive Oxygen Species (ROS) on Rat Aortic Smooth Muscle (RASM)

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Key words: Activated neutrophils – Reactive oxygen species – Rat aorta

This project was supported by the grant number APVV-51-017905.

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Introduction ROS are known to be involved in progression of various cardiovascular diseases. Their production is frequently associated with local inflammation and respiratory burst of polymorphonuclear leukocytes (PMNs). Smooth muscle cells may express inflammatory mediators and induce neutrophil activation and migration. Moreover vascular endothelial cells are key participants in the development of inflammatory-mediated injury. While resting PMNs generate NO, their activation in the inflammatory process as well as in response to arachidonic acid, PMA, fMLP, etc. leads to production and release of superoxide anion radical ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2).

The aim of the present work was to compare the effects of fMLP activated isolated peritoneal neutrophils with those of different ROS ($O_2^{\bullet -}$, $^{\bullet}OH$ and H_2O_2) on RASM.

Methods The experiments were performed on rings from isolated thoracic segments of RASM. Neutrophils were acquired from the peritoneal cavity of guinea-pigs. H_2O_2 (10^{-5}–10^{-3} mol/l) was added as pure chemical, $O_2^{\bullet -}$ was

generated by 10^{-4} mol/l xanthine plus 0.1 IU/ml xanthine oxidase, and $^{\bullet}OH$ was produced by 10^{-4} mol/l $FeSO_4$ plus 1,5 x 10^{-4} mol/l H_2O_2.

Results and Conclusions The first series of the experiments were performed to analyze the influence of various precontractions. In KCl (5, 15, 30, 100 mmol/l) precontracted RASM the effects of H_2O_2 (10^{-5}–10^{-3} mol/l) was dependent on the KCl induced initial tone. Contraction dominated at low and relaxation at high initial KCl evoked tone. Both the H_2O_2 induced contraction (which was significantly enhanced by 0.5 mmol/l L-NAME and endothelium removal) and relaxation were catalase (CAT) sensitive. While in KCl (100 mmol/l) precontracted RASM the native neutrophils did not possess any effect, neutrophils (10^7/ml) activated by fMLP (10^{-7} mol/l) (ANT) elicited biphasic response (relaxation-contraction). The ANT induced response was similar to that evoked by $O_2^{\bullet-}$ and differed from the H_2O_2 and $^{\bullet}OH$ caused contraction-relaxation. In phenylephrine (PhE, 10^{-6} mol/l) precontracted RASM H_2O_2 and $^{\bullet}OH$ evoked biphasic change of the muscle tone (contraction-relaxation). In noradrenaline (NA) precontarcted tissues the ANT evoked initial contraction which was broken down by marked relaxation, probably due to oxidation and inactivation of NA with $O_2^{\bullet-}$.

Since the responses in PhE precontracted RASM were most invariable, the second series of experiments were accomplished on RASM precontracted by PhE. CAT (1000 IU/ml) with superoxide dismutase (SOD – 30 IU/ml) eliminated the action of ANT, while CAT alone reduced the contraction and SOD unmasked the ANT evoked relaxation in PhE precontracted tissues. Inhibition of CAT by 3-amino-1,2,4-triazole (0.1 mmol/l) and SOD by sodium diethyl-dithio carbamate (3 mmol/l) markedly reduced the contractile action of ANT. Nordihydroguaiaretic acide (10^{-5} mol/l) equally reduced the PhE and ANT induced contractions and the effects of $^{\bullet}OH$. Indomethacine (10^{-6} mol/l) did not affect the actions of H_2O_2, selectively impaired the contractile action of ANT and the relaxation induced by $^{\bullet}OH$, whereas accentuated the contraction and impaired the relaxation elicited by $O_2^{\bullet-}$. Stobadine (10^{-5} mol/l) which possesses antioxidant and α-adrenolytic properties reduced the contractions evoked not only by PhE, NA but also by ANT.

There are different endogenous sources of ROS which may affect function of vessels. Most frequently ROS are produced either by lipoxygenase, cyclooxygenase or by NADPH oxidase and myeloperoxidase of activated PMNs. Our study demonstrated that while native neutrophils do not influence, the fMLP activated ones evoke contraction, relaxation or biphasic response of RASM precontracted by various stimulants. ANT evoked changes in the RASM tone result most probably from $O_2^{\bullet-}$ production, which via its transformation to other ROS causes oxidative stress accompanied by alterations of muscle tone. These are due to direct or indirect (elimination of the protective role of the endothelium, interaction with NO itself or its production, or via prostanoid metabolism influencing cyclooxygenase and/or lipoxygenase pathways) action of ROS on RASM.

Role of Extracellular-signal Regulated Kinase (ERK) Pathway in PXR-mediated Valproic Acid-induced Activation of CYP3A4 Expression

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Keywords: Extracellular-signal regulated kinase (ERK) – Valproic acid – CYP3A4 – PXR

This project was supported by the grant GA UK No. 118708/C/2008 (M.B.) and GA ČR 303/07/0128 (P.P).

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Introduction Valproic acid (VPA) is a widely used drug for the treatment of epilepsy and bipolar disorder. In addition, the drug is nowadays tested as a potential anticancer drug. Valproic acid has been proven to affect numerous gene regulatory mechanisms including histone deacetylases (*HDACs*) and mitogen-activated protein kinase pathways (*MAPK*) such as extracellular-signal regulated kinase (*ERK*) signal transduction pathway.

As we showed earlier, valproic acid has the potential to up-regulate expression of cytochrome P-450 *CYP3A4* and P-glycoprotein (*ABCB1*) genes via constitutive androstane receptor (CAR) and pregnane X receptor (PXR) nuclear receptors and stimulate rifampicin-mediated induction of the gene. Inhibitory effects of valproic acid on *HDAC* and ERK1/2 activation are proposed to participate in the transactivation of the genes through PXR nuclear receptor.

In this study, we focused on involvement of the extracellular-signal regulated kinase (ERK) pathway in valproic acid-mediated transactivation of *CYP3A4* gene.

Methods The effect of valproic acid on *ERK1/2 MAPK* pathway and involvement of the pathway in PXR-mediated transactivation of *CYP3A4* gene was studied with pharmacological inhibitor of *ERK1/2* pathway, U0126, and employing small

interfering RNA (siRNA) in transient transfection gene reporter assay. Model ligand of PXR, rifampicin, was used in the experiments. Activation of *CYP3A4* promoter was determined using luciferase reporter assays in HepG2 cells and mRNA expression level monitored by real-time reverse transcriptase polymerase chain reaction (RT-PCR) in primary human hepatocytes.

Results and Conclusions We found that valproic acid activates *ERK1/2 MAPK* pathway employing Western blot analysis of phosphorylated *ERK1/2* protein in HepG2 cells (Figure A). We also found that U0126 activates *CYP3A4* promoter; however, it do not affect rifampicin-mediated activation of *CYP3A4* gene reporter. On the other hand, silencing of *ERK1/2* (*MAPK1/3*) suppressed both valproic acid- and rifampicin-mediated activation of *CYP3A4* promoter (Figure B and C). Our preliminary data suggest potential role of *ERK1/2* in valproic acid-induced PXR-mediated transactivation of CYP3A4 gene.

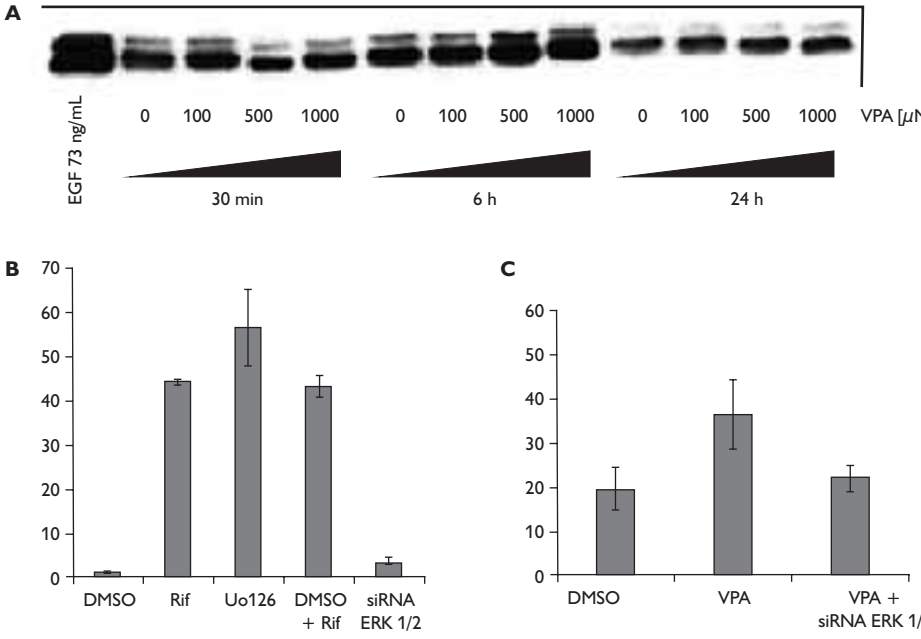

*Figure 1 – **A.** Valproic acid (VPA) activates ERK1/2 MAPK pathway in HepG2 cells. Time- and dose-dependent effect of VPA on ERK1/2 phosphorylation was studied with total protein extracts and western blotting analysis with anti-ERK-P (Thr202/Tyr204) antibody. **B.** and **C.** Effect of U0126 (10 μM) and siRNA ERK1/2 on rifampicin (Rif, 10 μM) or VPA (500 μM) – mediated activation of p3A4-luc gene reporter plasmid in HepG2 cells cotransfected with PXR expression plasmid.*

Transient transfection experiments have been performed as described before, in: *Drug Metab Dispos.* 2007/35.

Therapeutic Drug Monitoring of New Antiepileptic Drugs

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Key words: Antiepileptic drugs – Therapeutic drug monitoring

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Introduction TDM of the first and the second generation antiepileptic drugs (AEDs) has for many years served as a valuable tool in the treatment of epilepsy. For many of these drugs clinical effect correlates better with blood levels than with doses. TDM is also an effective instrument for detection of non-compliance because it occurs in one-third to one-half patients. Finally, the estimation of drug levels serves as an useful tool in patients on AEDs polytherapy, because many AEDs can induce (carbamazepine CMZ, phenytoin DPH, phenobarbital PB) or inhibit (valproic acid VPA) enzyme metabolism.

In the last few years the new generation of AEDs (the third) has been introduced into the treatment of epilepsy. When introduced to clinical praxis, TDM of the third generation AEDs was not considered necessary and the target therapeutic ranges were not defined. However the increase of number of patients treated with new AEDs has extended TDM also on this generation and gradually the therapeutic ranges have been establish for most of them.

Lamotrigine (LAM), topiramate (TOP) and levetiracetam (LEV) are the third generation AEDs measured in our department. The aim of this work was to point out the increasing significance of new AEDs in the treatment of epilepsy.

Methods LAM was determined by HPLC method simultaneously with other AEDs such as primidone (PD), PB, CMZ and DPH and two active metabolites, 2-ethyl 2-phenylmalonamide (PEMA) and carbamazepine- 10,11-epoxide (EPO). An ordinary reversed-phase system was used and a liquid-liquid extraction was performed. UV detection was carried out at a 220 nm.

GLC method involving liquid-liquid extraction with diethylether for simultaneous determination of TOP and other AEDs (PD, PB, CMZ, DPH) was developed and validated. Gas chromatograph equipped with Supelco capillary column (15 m × 0.25 mm; 0.25 μm film thickness) and flame thermoionic detector were used. TOP was analysed using on column derivatization (flash methylation) performed by trimethylphenylammonium hydroxide (TMAOH) at high temperature. Validation parameters are as follows: recovery (R) 91.0–105.5%;

coefficient of variation (CV) 3.5–9.3%. Linearity was in the range 0–25 mg/l (correlation coefficient was 0.998).

LEV was determined by HPLC method using reversed-phase system and liquid-liquid extraction with dichlormethane. Chromatography was performed on a microbore column 1 × 150 mm SGX C18 eluted with a mobile phase (water:methanol:acetonitrile 85:10:5). Detection was at 205 nm. Validation parameters are: R 101.4–102.5%; CV 1.4–6.6% and method was linear in the range 0–50 mg/l (correlation coefficient was 0.999).

Results and Conclusions LAM was introduced into TDM in our department in 2001, TOP in 2003 and LEV in 2005. The proportion of LAM, TOP and LEV on the total amount of AEDs measurements in the first year after their introduction into TDM was as follows: LAM 5.2%; TOP 1.7% and LEV 1.3%. As seen from the table 1 the number of measurements of third generation AEDs has significantly increased during their routine TDM: LAM 3.5 fold, TOP 5.1 fold and LEV 3.2 fold. The number of LAM measurements has even reached the number of the second generation AED carbamazepine.

Table 1 – Number of individual drugs measured at our department/year

year	LEV	TOP	LAM	PB	CMZ	DPH	PD	VPA	CLO	Σ AEP	% LEV	% TOP	% LAM
2001	–	–	331	332	2063	908	243	2082	382	6341			5.22
2002	–	–	616	272	1862	778	196	2350	449	6523			9.44
2003	–	127	828	302	2017	689	211	2603	531	7308		1.7	11.33
2004	–	509	952	248	1976	631	165	2746	665	7892		6.4	12.06
2005	99	488	1160	235	1799	538	127	2603	553	7602	1.30	6.4	15.26
2006	275	681	1255	311	1682	502	122	2594	587	8009	3.43	8.5	15.67
2007	340	705	1518	256	1551	417	109	2712	584	8192	4.15	8.6	18.53

CLO – clonazepam

Comparative Study of Natural Antioxidants – Silymarin and Resveratrol – in Thioacetamide-induced Liver Injury in Rats

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Key words: Antioxidants – Thioacetamide – Hepatotoxicity – Oxidative stress

This work was supported by the Grant MSM ČR No. 0021620819 and by the Specific Research of Charles University – Faculty of Medicine in Pilsen.

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Introduction Silymarin (SLM) is a mixture of flavonolignanes from the fruits of *Silybum marianum* with hepatoprotective properties. Resveratrol (RSV), a natural polyphenol present in grapes of *Vitis vinifera*, is known for its antioxidant and antiproliferative effects. Thioacetamide (TAA) is a hepatotoxic compound used in experimental work in hepatology. No study comparing the effects of silymarin and resveratrol in thioacetamide intoxication was published.

Aim The study is focused on the protective effect of natural antioxidants, silymarin and resveratrol, in thioacetamide-induced liver injury in rats.

Methods Male Wistar rats (150±10 g b.w., Velaz Prague) were randomly divided into 6 groups (n=7/8) with free access to diet and tape water. Animals were treated orally with silymarin (175 mg/kg b.w.) or resveratrol (10 mg/kg b.w.), dispersed in 0.5% methylcellulose, once daily for five days. On the 3rd and 4th day, thioacetamide (150 mg/kg b.w., i.p.) was administered one hour after SLM or RSV application. At the 24h after the last dose of antioxidant, animals were sacrificed by decapitation. The liver tissue and blood (serum) were collected for biochemical analysis. The experimental treatment protocol was approved by the local Animal Care and Use Committee. In the liver homogenates, the level of reduced glutathione (GSH), the lipid peroxidation (LP, expressed as malondialdehyde production formed in thiobarbituric acid reaction), the activities of glutathione-peroxidase (GSH-Px) and catalase (CAT), were estimated. The serum alanine-aminotransferase (ALT) and aspartate-aminotransferase (AST) levels were determined. Student's unpaired t-test was used to analyze mean differences between experimental groups.

Results The administrations of thioacetamide resulted in the increase of LP (to 189%; $p<0.001$) and GSH content (to 114%; $p<0.05$) and in the decrease in activities of GSH-Px (to 77%; $p<0.001$) and CAT (to 71%; $p<0.001$) in the liver tissue, compared to control group. The serum aminotransferases activities were significantly elevated in comparison to control group (ALT to 245%; $p<0.001$ and AST to 211%; $p<0.001$). The treatment of silymarin significantly enhanced the GSH-Px ($p<0.05$) and CAT ($p<0.05$) activities; the treatment of resveratrol ameliorated the hepatic LP level ($p<0.005$) and the serum ALT activity ($p<0.05$) compared to TAA-only treated group. RSV and SLM given alone caused a higher activity CAT ($p<0.001$), SLM diminished the GSH-Px level ($p<0.001$) compared to control group.

Table 1 – Effects of TAA and antioxidants on important indicators of the experiment

	LP [nmol MDA/g]	GSH-Px [μmol NADPH/min/g]	CAT [*k*/g]	ALT [U/L]
CONTROL	29.4 ± 6.8	29.2 ± 1.36	51.3 ± 4.8	51.5 ± 6.8
SLM	34.4 ± 2.5	25.7 ± 0.86**	65.5 ± 7.13**	55.8 ± 6.0
RSV	33.3 ± 2.4	27.9 ± 2.42	54.7 ± 3.8*	56.9 ± 4.1
TAA	55.7 ± 6.8**	22.5 ± 1.81**	36.5 ± 3.5**	126.4 ± 32.1**
TAA+SLM	50.6 ± 6.2	25.1 ± 1.66$^{\#}$	42.3 ± 5.2$^{\#}$	110.6 ± 24.9
TAA+RSV	41.2 ± 8.0$^{\#\#}$	23.6 ± 2.76	37.9 ± 7.8	92.5 ± 16.5$^{\#}$

*p<0.05 and **p<0.001 vs control; $^{\#}$p<0.05 and $^{\#\#}$p<0.005 vs TAA

Conclusions The thioacetamide-induced liver injury was demonstrated by the elevation of hepatic LP and serum aminotransferases activity and by lowering of the activities of GSH-Px and CAT. The protective effect of silymarin was exerted by enhancing of GSH-Px and CAT activities in the liver of TAA-exposed rats. The hepatoprotective action of resveratrol appears from ameliorating of LP level and serum ALT activity of TAA-exposed animals. SLM and RSV exert probably different mechanism of action. Both antioxidants studied in this experiment may serve as protective agents in acute thioacetamide-induced liver damage.

Potential Hepatoprotective Effects of Resveratrol Pretreatment on *tert*-butyl-hydroperoxide Induced Toxicity in Immobilized Perifused Hepatocytes

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Key words: Resveratrol – Tert-butyl-hydroperoxide – Liver – HO-1 – NOS-2

Supported by research grants IGA MZ ČR NR/9379-3/2007 and VZ MSM ČR No. 0021620807.

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Introduction Resveratrol, a naturally occurring antioxidative agent, is a polyphenolic compound related to the stilbene class, found in some trees, in a few flowering plants, in peanuts and in grapevines. Silymarin (milk thistle, Silybum marianum) is a the well known naturally occurring substance of plant origin with documented hepatoprotective effect which is used as a standard liver protector since it exhibited significant activities *in vitro*, *in vivo* and in clinical trials.

The purpose of this work was, therefore, to study the effects of resveratrol (RES) compared to silymarin (SM) pretreatments on *tert*-butylhydroperoxide (tBH) toxicity model in hepatocytes including apoptotic/necrotic markers, heme-oxygenase 1 (HO-1) and inducible nitric oxide synthase (NOS-2) expression. The mutual cross talk between NO and CO signaling molecules in apoptosis and necrosis is also being evaluated.

Methods Hepatocytes in perifused immobilized agarose threads (5 h) were used as a cellular system. Cell apoptosis was estimated morphologically and hepatocyte viability and functionality were evaluated by ALT and urea synthesis. Urea and ALT concentration in the medium samples were measured spectrofotometrically using customized diagnostic kits according manufacturer´s instruction. Nitric oxide (NO) and carbon monoxide (CO) involvements were examined carefully. Expressions of inducible NOS-2 and HO-1 were measured by real time RT-PCR. Medium NO_2, a stable end-product of NO oxidation, was determined spectrofotometrically by using Griess reagent and CO levels/HO-1 activity were measured by gas chromatography with UV detection. Using detection of apoptosis by Annexin V in combination with propidium iodide (PI) for estimation of nuclear morphology enabled the evaluation of the proportion of apoptotic and necrotic cell populations at the end of 5 h perifusion and 48 h culture periods. Apoptotic hepatocytes were discriminated by the green fluorescence that was caused by Annexin-V-FITC binding on phosphatidylserines present at the cell membrane surface. PI, which gives red fluorescent, was used to stain the nuclear DNA of all fixed cells. The statistical significance of differences of mean scores was determined using unpaired two-tailed Student's *t*-test for comparison between two groups or by one-way analysis (ANOVA) of variance followed by Bonferroni Multiple Comparisons test. P-value less than at least 0.05 was considered to be statistically significant (see Results). Data were expressed as means ± SEM (standard error of mean) of at least three (4–12) independent experiments with blind samples as the media background.

Results Resveratrol and silymarin reduced tBH induced hepatocyte toxic effects in short term experiments (5 h) as measured by significant reduction in ALT

increase produced by tBH. Both inducible nitric oxide synthase (NOS-2) and hemoxygenase-1 (HO-1) gene expression were increased by tBH and reduced by both RES and SM pretreatments. These effects correlated with the levels of NO and CO. Morphologically, there were ameliorations in both apoptotic and necrotic markers under RES treatment and were similar to biochemical findings. In addition, RES improved hepatocyte stability in both cellular systems.

Conclusions

1. A significant hepatoprotective effects of the studied compounds was demonstrated by using a short term study in vitro model (perifused immobilized hepatocyte bioreactor).
2. RES per se stabilized the immobilized hepatocytes.
3. RES 10 μM shows the similar effect like SM 500 μM.
4. Important signals which may contribute to the effects of RES and SM against apoptosis or necrosis observed in this study are NO, CO and HO-1, NOS-2.
5. Under the present model of hepatotoxicity (tBH), low expressions of the NOS-2 and HO-1 enzymes shows that the effects of resveratrol and silymarin did not depend on up regulation of these proteins.
6. Resveratrol and sylimarin ameliorative effects on tBH hepatocyte toxicity are comparable and should be re-evaluated in vivo experimental conditions.

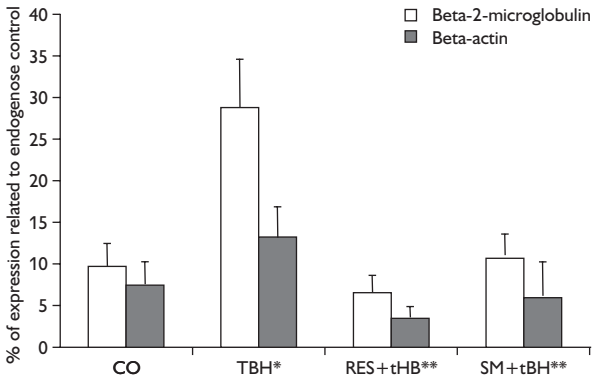

*Figure 1 – This graph demonstrates that both resveratrol and silymarin highly significantly reduced tBH-induced increase in HO-1 expression related to beta-2-microglobulin and beta-actin as endogenous controls. CO pure medium – control, tBH tert-butylhydroperoxide 1 mM, RES resveratrol 10 μM, SM silymarin 500 μM, *significant to control, **significant to tBH. Means ± SEM, n = 4–5.*

Evaluation of Polymorphisms of XRCC1 (Arg399Gln) and MDR1 (C3435T) and their Correlation with Therapeutic Efficacy and Haemotoxicity in Patients with Breast Cancer

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Key words: Polymorphisms – XRCC1 – MDR1 – Breast cancer

This research project was supported by the grant MZ SR No. 2005/46-VOUKE-01 and by grants VEGA SR 1/2282/05 and VEGA SR 1/3372/06.

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Introduction Inter-individual variability in therapeutic drug responses and drug toxicities is a major problem in cancer chemotherapy. Pharmacogenetic pretherapeutic screening of single nucleotide polymorphisms (SNP) in relevant genes, which encode for proteins that interact with anticancer drugs, may lead to identification of specific populations predisposed to poor drug responses and drug toxicity. Pharmacogenetics for individualized cancer chemotherapy is, therefore, an important area of investigation.

The main purpose of this study was to evaluate correlation of two pharmacogenetic factors (XRCC1 Arg399Gln and MDR1 C3435T polymorphisms) with therapeutic efficacy and haemotoxicity in breast cancer patients treated by alkylating agents and anthracyclines using in neoadjuvant and/or adjuvant chemotherapy. We also examined the role of these polymorphisms as genetic indicators of susceptibility to breast cancer.

Methods In our study we identified the XRCC1 Arg399Gln and MDR1 C3435T polymorphisms in breast cancer patients (n=113) and healthy subjects (n=113). Genomic DNA was extracted from peripheral blood lymphocytes using standard extraction method. Polymerase chain reaction-restriction fragment length polymorphism was used for detection of single nucleotide polymorphisms. Therapeutic response was assessed using Response Evaluation Criteria in Solid Tumour guidelines and haemotoxicity was classified according to WHO criteria. Statistical analyses were performed using the χ^2-test or Fisher exact test, when was needed. Progression-free survival was estimated using the Kaplan-Meier method and compared by log-rank test.

Results and Conclusions First XRCC1 Arg399Gln polymorphism was investigated. Comparison of genotype and allele frequencies between controls and patients with breast cancer showed no significant difference. We also evaluated the progression-free survival, finding significant result (log-rank, Mantel-Cox test,

p=0.009) and therapeutic efficacy after neoadjuvant therapy (Fisher exact test, p=0.047). We did not find any significant difference between particular genotypes and occurrence of haemotoxicity.

The MDR1 C3435T polymorphism was the second analysed SNP. There was significant difference in genotype and allele distribution between breast cancer patients and control group; when in the patient group, T allele was higher than controls ($p<0.05$). Progression-free survival and the presence of haemotoxicity were not found to be statistically significant between particular genotypes. However, there was significant result observed in therapeutic outcome after neoadjuvant therapy (Fisher exact test, p=0.023).

In conclusion, our preliminary data demonstrate increased risk for development of breast cancer in T allele carriers of MDR1 C3435T polymorphism, possible impact of the XRCC1 Arg399Gln polymorphism on progression-free survival and influence of both investigated polymorphisms on therapeutic efficacy after neoadjuvant therapy in breast cancer patients treated by alkylating agents and anthracyclines.

Importance of P-gp and Bcrp for Detoxication Role of Placenta

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Key words: Breast cancer resistance protein – P-glycoprotein – Placenta – Drug transport – ABC drug efflux transporters

This project was supported by the grant No. 119007 C 2007 FaF GA Charles University in Prague, and by the grant No. 1A/8696-4 of the MZ ČR.

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Introduction ABC (ATP binding cassettes) drug efflux transporters P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP) have been shown to be highly expressed in many physiological tissues. In the placenta, they contribute to the protection of developing fetus against potentially harmful substances from mother. Treatment of some diseases during pregnancy is often unavoidable and must be conducted even without a precise knowledge of transplacental pharmacokinetics (PK) of the drugs and their potential risk for the fetus. Intensive study of mechanisms influencing the transport of drugs across placenta is important for optimization of pharmacotherapy in pregnancy. The

intention of our study was to describe the role of P-gp and Bcrp in transplacental PK of rhodamine 123 (Rho123; substrate of P-gp), BODIPY FL Prazosin (BP; substrate of both P-gp and BCRP) and glybenclamide (Gly; BCRP substrate) employing the model of dually perfused rat placenta.

Methods The method of dually perfused rat term placenta was carried out as described previously. Two types of perfusion systems (open or fetal recirculation) were used in this study. To study maternal-to-fetal and fetal-to-maternal clearances, substrates were added to the maternal or fetal reservoirs, respectively and their concentrations were measured in the fetal effluent. To study feto/maternal concentration ratio at steady state, both maternal and fetal sides were infused with equal, non saturating concentrations of substrate and the fetal perfusate was recirculated for 60 min. GF120918 or Fumitremorgin C were used as inhibitors of P-gp and/or Bcrp.

Results and Conclusions For all substrates used in this study (Rho123, BP and Gly) maternal-to-fetal clearances were significantly lower than those in the opposite direction (fetal-to-maternal clearances were 11.4 and 11.6-fold higher, respectively). Addition of a P-gp and BCRP inhibitor GF120918 decreased this asymmetry to 4.6, 2.4 and 2.1, respectively. Moreover, we demonstrate the potential of these transporters to pump their substrates against a concentration gradient from fetus to mother. Ratios of feto/maternal concentrations 60 min after the beginning of fetal perfusate recirculation were 0.32 for Rho123, 0.47 for BP and 0.20 for Gly. Inhibition or saturation of the transporters significantly increased these ratios. Our results show that both P-gp and Bcrp are able to hinder the transport of their substrates from maternal to fetal circulation. In addition, their ability to actively remove substances already present in fetal circulation has been observed. As BP is a substrate of both transporters, we expected that its transplacental PK will be affected more than those of the other two compounds. However, this was not the case; the ratio of fm-to-mf clearance was much lower in the case of BP compared to the other tested substances. It seems plausible, that other factors, such as physical-chemical properties, are important in determining the net transplacental PK.

Structure and Dynamics of Drug Targets: Introduction and Focus on Receptors and Transporter Proteins

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Key words: Molecular structure – Molecular modelling – Molecular dynamics

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Introduction and Aim The DNA sequencing of the human genome led to identification of many previously unknown proteins which may represent potential drug targets. In order to fully understand the functional mechanisms of a known or novel potential drug target, it is crucial to know its 3-dimensional molecular structure. This may be determined experimentally by X-ray crystallography, NMR spectroscopy or electron microscopy, and computationally by structural bioinformatics and molecular modelling. When the structure of a drug target is known, computer programs can be used to predict ligand-target binding affinities and to search for novel drug candidates.

Drug targets may be classified as
- Enzymes
- Membrane proteins
  - Receptors (G protein coupled; ligand gated ion channels; kinase linked)
  - Ion channels and transporters
- Nuclear receptors.

More than 95 % of current drug targets are proteins. Modelling of drug-target interactions therefore usually implies modelling of a 3-dimensional protein structure. Such models are based on a 3-dimensional template protein structure. The accuracy of protein models constructed by such methods depends on how accurately the template protein structure has been determined, the structural and functional resemblance between the template protein and the modelled protein, and how well their amino acid structures may be aligned.

Ion channels, active carrier proteins (transporters) and G protein coupled receptors (GPCRs) are membrane proteins which represent important classes of current and potential new drug targets. Membrane proteins have proven extremely difficult to purify and crystallize due to their amphipathic surface, with a hydrophobic area in contact with membrane phospholipids and polar surface areas in contact with the aqueous phases on both sides of the membrane. Still, a small but increasing number of membrane proteins have now been crystallised and their structure determined at atomic resolution. We have used such structures as templates for molecular modelling of receptors and transporters which represent molecular targets for drugs within various therapeutic areas.

Studies of the molecular dynamics of biologically active molecules have demonstrated that such molecules are indeed as alive as the organisms in which they act. A rigid-structure "lock and key" concept does not adequately describe drug-target interactions, since all such functional mechanisms require motion at the

molecular level. Computer simulation of proteins and other macromolecules, which is the most widely used method to study their molecular dynamics, requires relatively high-performing computers.

Conclusions Our molecular modelling and simulation studies have demonstrated that

- Transporters and G-protein coupled receptors have a dipolar electrostatic structure: Negative outside and positive inside the cell membrane. Electrostatic charges pull drugs and neurotransmitters, which are protonated and positively charged at pH 7.4, into the primary receptor/transporter binding site.
- Receptors and transporters have flexible structures and their function requires motion: In order to explain their molecular mechanisms, both the target protein and the ligand must be regarded as highly flexible entities.
- Ligand interactions may lead to changes in
  – molecular conformations
  – electrostatic fields of functionally important protein domains.
- High-resolution crystal structures used as templates provide more accurate protein models than those constructed from low-resolution protein templates.
- Previous 3-dimensional GPCR models have been corroborated by reported crystal structures of rhodopsin and a $beta_2$ adrenergic receptor.

The Cost of Type 2 Diabetes Mellitus in Czech Republic

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Key words: Diabetes – Type 2 – Cost of illness – Direct cost

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Introduction Diabetes mellitus is chronic long-life disease with increasing incidence and prevalence. It is a public health issue of significant economic importance because of the chronic nature and the serious complications associated with long disease duration. The CODE-2 study in 8 countries published in 2002 was the first study measuring the cost of type 2 diabetes in Europe. In Czech Republic is need for studies that examine the healthcare recource utilisation and medical cost of diabetes.

Aim of the study The aim of the study was to describe the direct medical cost for average patient with type 2 diabetes and for whole population of diabetics in Czech Republic using "bottom-up" costing approach.

Methods The data was collected in ambulatory diabetologists by specific questionnaire. The questionnaire was used to collect information on direct medical resource utilisation and clinical data based on diabetologist-held medical records. Data were collected between October nad December 2007 and covered a minimum period of 6 months, retrospectively. Estimates of healthcare utilisation and costs were projected for a 12-month period. The overall direct healthcare costs were calculated by multiplying the quantities of the resource used with the unit reimbursement of each resource by insurance company.

Results *Demographics:* During the collection phase of the study 495 patient records were gained. The mean age was 63 years, with 52% of men and 48% of women. The average time from diagnosis of diabetes was 10 years. The average HbA_{1C} values were 6.0%, body weight 92.6 kg for men and 82.0 kg for women, body mass index (BMI) was 29.9 for men and 31.1 for women and the average blood presure was 141/80 mm Hg. The prevalence of diabetic complications is shown in table 1. At least one microvascular complication is present in 49.29% and at least one macrovascular complication in 44.24% of type 2 diabetic patients.

Healthcare utilisation: According to the type of treatment 10.51% of patents were treated with diet and exercise, 49.9% were on oral antidiabetic drugs and 39.6% were on insulin therapy. Hypolipidemic drugs were present in 63.4% and antihypertensive drugs in 82.8% of patients. The majority of diabetic patients visited ambulatory diabetologist (98.99%, mean number of visits 2.3), general practitioner (91.31%, mean number of visits 3) or other ambulatory specialist

Table 1 – Prevalence of complications in type 2 diabetic patients in Czech Republic

Complication	Percentage of patients
Microalbuminuria	18.38%
Proteinuria	8.48%
Dialysis	1.41%
Retinopathy	24.85%
Neuropathy	41.82%
Coronary artery disease	24.04%
Stroke/TIA	9.29%
Diabetic foot	4.65%
Stable angina	25.25%

Table 2 – Division of the annual costs (CZK) per person according to cost components

Type of costs	Cost per person (CZK)	Share of total (%)
Inpatient care	15 824	61%
Outpatient care	1 741	5%
Insulins	3 974	15%
Oral antidiabetic drugs	1 026	4%
Hypolipidemics	2 191	9%
Antihypertensives	1 102	4%
Total	25 858	100%

(75.4%), mainly ophtalmology, cardiology or neurology in last 6 month. In the same time period 16.16% of patients were hospitalized with average length of stay 13.68 days (1.2 days in intensice care unit bed).

Direct medical costs: The final distribution of the direct medical costs in type 2 diabetic patients is shown in Table 2. The mean direct medical expenditures for one patient is 25 858 CZK per year. In the ambulatory care the majority is spent for diabetiology care and in hospital setting for internal medicine and cardiology.

Conclusion In conclusion, this study provides a comprehensive resource use and cost analysis for representative sample of patients with diabetes type 2 using a "bottom-up" approach. Based on data from Institute of Health Information and Statistics of the Czech Republic in 2006 there were 678 760 type 2 diabetic patients in our country. It it roughly 17.5 bilion CZK per year, counting approximately 20% of total healthcare spending in Czech Republic.

What Blood Sample Time is the Most Suitable for the Determination of Metoprolol Metabolic Ratio?

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Key words: CYP2D6 – Metoprolol – Serum metabolic ratio

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Introduction: Cytochrome P450 2D6 (CYP2D6) is one of the most important enzymes involved in the metabolism of about 25% of all commonly prescribed drugs. The activity of CYP2D6 shows a high degree of interindividual variability mainly caused by genetic polymorphisms. However, genotyping alone is not sufficient to accurately predict an individual´s CYP2D6 activity, as this is also influenced by environmentally factors. Phenotyping offers the possibility to determine the actual enzymatic activity. It is based on administration of a probe drug – a compound that is predominantly metabolised by CYP2D6. The enzymatic activity is assessed by a metabolic ratio (MR) of a parent compound/metabolite in urine or plasma. Metoprolol serves well as a probe for CYP2D6. Several authors have found close correlation among the oxidative capacities for metoprolol and other CYP2D6 probe drugs, such as debrisoquine, sparteine and dextromethorphan. A one-point plasma MR of metoprolol has been used to determine the CYP2D6 metabolic activity. However, different single blood samples times have been used to determine the MR.

The aim of this work was to examine the correlation between metoprolol MRs in different single blood samples and to assess the best blood sample time.

Methods 14 patients attending outpatient department for the treatment of hypertension at University Hospital in Ostrava were included, age 64.5 ± 12.1 years. Patients were recruited if they were on metoprolol therapy for at least 1 month and there were no dosage changes for at least one week. They were retained in the ward for a half-a-day study procedure. Baseline data including renal function test and liver function test were obtained either on the study day or within 3 months before the study day.

Venous blood samples (4.9 ml each) were drawn into a neutral tube before and at 1, 3 and 4 hours after metoprolol intake (Betaloc SR or Betaloc ZOK). Serum concentrations of metoprolol and α-hydroxymetoprolol were measured by means of HPLC with fluorescence detection at 230–300 nm. The column was Supercosil LC-18 (15 × 3.5 μm). The mobile phase consisted of acetonitrile:methanol:water: TEA (30:10:60:0.04), pH 3.4. The flow rate was 0.05 ml/min. IS-pindolol was used as the internal standard. Prior to analysis, the drug was separated from 200 μl serum with 50 μl 1M NaOH. 1.5 ml of dichloromethane was used for extraction. After evaporation, the analyte was dissolved in 20 μl of methanol and 50 μl of water. 20 μl was injected. The metoprolol/α-hydroxymetoprolol MR was calculated. Spearman's rank correlation test was used for evaluating the relationship between metoprolol MRs.

Results and conclusions The metoprolol MRs at individual blood sample times (before metoprolol intake, at 1, 3 and 4 hours postdose) are presented in Table 1.

Table 1 – Metabolic ratio (MR) before metoprolol intake (MR 0H) and at 1, 3, 4 hours postdose

preparation	MR 0H	MR 1H	MR 3H	MR 4H
Betaloc SR	0.50	1.12	1.49	1.32
Betaloc SR	0.31	0.50	0.57	0.50
Betaloc SR	0.55	5.21	4.24	4.21
Betaloc SR	0.52	0.80	1.50	1.22
Betaloc SR	0.25	0.37	0.68	0.68
Betaloc ZOK	0.28	0.26	0.40	0.33
Betaloc SR	2.73	3.01	3.53	3.24
Betaloc ZOK	1.84	1.99	2.36	2.36
Betaloc SR	0.15	0.40	0.41	0.36
Betaloc SR	0.36	0.55	1.14	1.17
Betaloc SR	0.16	0.41	0.39	0.37
Betaloc SR	0.38	1.27	1.56	1.71
Betaloc SR	0.35	0.78	0.81	0.70
Betaloc SR	0.24	0.83	1.22	1.11

Although the correlations between MRs were statistically significant, it is obvious from the table that the most distinctive differences were observed with mean serum MR before metoprolol intake 0.62 ± 0.71. The mean metoprolol MRs at 1, 3 and 4 hours postdose were 1.25 ± 1.31, 1.45 ± 1.14 and 1.38 ± 1.12. The lowest difference in MRs was found between MR 3 hours postdose and MR 4 hours postdose with the strongest correlation (r_s = 0.9780, P <0.0001). These results suggest that one-point serum sample either 3 hours postdose or 4 hours postdose is convenient for the serum MR metoprolol determination.

Molecular Genetic Identification of the Major CYP2D6 Alleles and Utilization in Psychiatric Treatment

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Key words: Genotype – CYP2D6 – HRM – Real-Time PCR – Psychiatry

Supported by research project MSM ČR 40021622404.

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Introduction The most frequently applied antidepressant such as serotonin selective reuptake inhibitors and dual antidepressants are metabolized by the p450 isoenzyme system.

Our attention is focused on this isoenzyme CYP2D6, which is co-responsible for metabolization of not only antidepressants, but also some antipsychotics, beta blockers and antiarhythmics. The gene CYP2D6 (22q13.1) that encodes this enzyme is highly polymorphic and shows a great inter-individual and inter-ethnic variability.

The polymorphisms leads to different individual responses following drug administration and increased risk of adverse reactions or the lack of the therapeutic response.

The Caucasian population has been grouped in according to the enzymatic activity as (i) poor metabolizers, who are slow to degrade CYP2D6 metabolites and who are exposed to a greater incidence of side effects of therapy

(ii) intermediate metabolizers are the subgroup between poor and effective metabolizers (iii) effective metabolizers, in which the metabolism follows the presupposed mechanism and (iv) ultrarapid metabolizers, who don't show adequate clinical response to common drug dosages.

There are studied influences of polymorphisms to the Paroxetine treatment in Department of Medical Genetics and Psychiatric clinic.

Methods The methodical approach is based on nested long PCR. Subsequently 5.2 kb PCR product is sequenced. These standard methods are combinated with modern and effective methods – Real Time PCR and High Resolution Melting using the Light Cycler 480 System. Recently is possible to detect the most frequent null alleles 3* 4* 6* 7* and 8* using the specific fluorescent labeled probes. Up to 99% of poor metabolizers in the Caucasian population can be detected with genetic testing for only 5 alleles (plus allele 5*, which can be detected by using the Sybr green). The High Resolution Melting (HRM) has been performed for the most frequent SNP's in CYP2D6 gene – 100 C>T, 2850 C>T and 1846 G>A. Described HRM is very fast cheap and reliable method for pre-genotyping. Heterozygous samples are readily distinguished from homozygous and wild-type samples with used dyes.

Results and Conclusions This paper provides an overview of current technologies available for assessing CYP2D6 polymorphisms in Department of Medical Genetics.

How is the Adherence to the Guideline in High-risk Cardiovascular Patients in Slovakia?

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Key words: Adherence to the guideline – High-risk cardiovascular patients

This project was supported by the grant number HOPE TOO study (The Heart Outcomes Prevention Evaluation – The Ongoing Outcomes Study), FaF UK/017/2004 – G 203, UK/260/2005, 823/2000-16.

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Introduction Slovakia exhibits alarmingly high cardiovascular mortality in European Union. One of the probable causes could be treatment not in line with the guideline. Standard therapeutic procedures (STP) provide a guideline for therapy of patients in Slovakia, including the cardiovascular ones. Adherence of physicians to the STP was not tested in Slovakia until now.

The aim of our study was to evaluate how many high-risk cardiovascular patients are not adherent to the guideline.

Methods We followed 849 high-risk patients (435 males and 414 females, age >55 yrs mean – 67.2 yrs, 55–88 yrs), who were enrolled into prospective trial HOPE-TOO during five years (2000–2005). Main inclusion criterion was presence of symptomatic coronary artery disease (CAD) [MI (45.5%), unstable angina pectoris (7.3%), stroke (17.8%), peripheral vascular disease (11.3%)] or diabetes mellitus (DM) (62.5%) associated with with one of additional risk factors: arterial hypertension (AH) (79.9%), hypercholesterolemia (66.1%) or smoking (45.9%). Patients with congestive heart failure (CHF) (NYHA >1) on entry were excluded. Other criteria excluded conditions and diseases influencing homocysteine metabolism or other important non-cardiovascular diseases expected to limit compliance. HOPE-TOO trial protocol did not analyse concomitant therapy in detail and Steering committee agreed to perform this follow-up in our centre. Concomitant therapy was controlled on every yearly visits. Patients were asked to bring with all prescribed drugs and to explain their intake. Drug was excluded from records only if patient reported prescription of one package and intake less then one month. Antibiotic therapy was not considered. No patient was lost of follow-up. General practitioners and care providing specialists were not influenced in their therapeutic decisions, but they were recommended to follow STP guidelines. Patients were defined as adherent to the STP, if they took at least one of the STP recommended drugs. STP recommended therapy for selected diagnosis is in Table 1.

Table 1 – STP recoomended therapy for selected diagnosis

Diagnosis	STP recommended therapy
Stroke	Ca channel blockers or ACEI
AH	BB or diuretics or Ca channel blockers or ACEI or AT1 blockers
CAD	Nitrates or trimetazidin or BB or Ca channel blockers or antiplatelet agents
AH+DM	BB1sel or BKV or ACEI or diuror insulin or PAD
CAD + CHF	ACEI or BB or digoxin or diuretics or Ca channel blockers or nitrates or AT1 blockers
MI	BB or ASA or other antiplatelet agents

AH – arterial hypertension, CAD – coronary artery disease, DM – diabetes mellitus, CHF – congestive heart failure, MI – myocardial infarction, Ca channel blockers – calcium channel blockers, ACEI – angiotensin converting enzyme inhibitors, AT1 blockers – sartans, BB – betablockers, ASA – acetylsalicylic acid

Results and Conclusions In table 2 is presented percentafge of STP nonadherent patients. Even with very weak criteria for adherence, we found 20% of patients after stroke not treated neither with ACEI nor with calcium channel blockers, 8% of known hypertonics without any antihypertensives and 8% of CAD patients lacking drugs recommended by STP. Significantly higher mortality was found in patients with stroke, res. with arterial hypertension who were nonadherent to the therapy [(56.6% (res. 43.2%) for lacking calcium channel blockers and 27.8% (res. 21.6%) for lacking ACEI).]

High risk cardiovascular patients are often treated discordantly to Standard Therapeutical Procedures. Nonadherent patients (especially after stroke and with arterial hypertension) exhibited alarmingly higher mortality.

Table 2 – Percentage of nonadherent patients per group

year	0 (%)	1 (%)	2 (%)	3 (%)	4 (%)	5 (%)	% average per year
stroke	28.9	20.3	24.8	18.3	16.0	17.8	21.0
CAD	7.4	7.8	9.5	9.0	15.9	7.8	9.6
AH	8.7	7.7	10.8	8.8	8.4	5.9	8.4
AH+DM	4.4	3.9	6.6	5.9	5.6	4.0	5.1
CHF	1.0	1.4	0.9	2.4	3.2	0.8	1.6
MI	1.0	1.9	2.9	1.9	5.5	1.2	2.4

CAD – coronary artery disease, AH – erterial hypertension, DM – diabetes mellitus, CHF – congestive heart failure, MI – myocardial infarction

New Ultrashort-acting Hypotensive Compounds

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Key words: Hypotensive effect – Plasma esterases – Ester functional group – Compound 44Bu – Compound 444

Financial support by the grant GA ČR No. 305/06/0863 is gratefully acknowledged.

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Introduction The ultrashort-acting beta blockers are parenteral agents that are used in special clinical settings where immediate beta adrenergic blockade is warranted, e.g. in patients with unstable angina, myocardial infarction, atrial fibrillation or flutter and supraventriclar tachycardia.

Two isomers of compound 444 – R and S, compound 444, compound 44Bu and esmolole were tested in this experimental work. Compound 44Bu, 444 and its isomers were synthesized as ultrashort-acting beta blockers at the Department of Chemical Drugs of the Faculty of Pharmacy of Veterinary and Pharmaceutical Sciences in Brno. The advantage of this agents should be in the immediate onset of action after administration, possibility of dose titration, very short duration of action and rapid offset of the action. The synthesized compounds are derivatives of arylcarbonyloxyaminopropanoles containing metabolically unstable ester functional group which is rapidly hydrolyzed by esterases in red blood cells, plasma and liver esterases.

Methods The aim of this work was to pharmacologically evaluate the effect of above mentioned agents by way of non invasive method on systolic blood pressure in rats. The experiment was performed *in vivo* with 42 male Wistar laboratory rats. All the tested agents were administered at the unified dose of 2.5 mg/kg of body mass. The tested group was divided into 6 subgroups: Group 1 (n=8) was administered agent 44Bu, Group 2 (n=8) agent 444, Group 3 (n=7) isomer R of agent 444 (444R), Group 4 (n=7) isomer S of agent 444 (444S), Group 5 (n=6) esmolole and Group 6 (n=6) placebo. The tested doses were administered into *vena jugularis* and the values of systolic blood pressure were monitored for 20 minutes following the administration. For systolic blood pressure non invasive monitoring a "Non-Invasive Blood Pressure Monitor" by Columbus Instruments company was used. The tested doses were compared with placebo.

Results and Conclusions The agent 44Bu caused statistically significant decrease ($p<0.01$) in systolic blood pressure compared to the placebo 12 minutes after administration, agent 444 9 minutes, isomer R of agent 444 11 minutes, isomer S of agent 444 12 minutes and esmolole 16 minutes.

The deepest decrease of systolic blood pressure was caused by agent 444 in the 3rd minute – 71.6%, esmolole caused decrease 76.4% in the 7th minute, agent 444S 78.7% in the 2nd minute and agent 44Bu 81.6% in the 2nd minute.

All the tested agents caused in trial by way of non-invasive method hypotensive effect which started immediately after administration and the hypotensive effect was ultrashort.

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Amiodarone Modulates Excretion of Conjugated Bilirubin in Rats.

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Key words: Amiodarone – Conjugated bilirubin – Mrp2 – Bbiliary excretion – Renal clearance

This study was supported by a grant from MSM ČR No. 1P05OC061 – COST B25.001.

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Introduction Amiodarone, a widely used antiarrhythmic agent, is known to produce drug-drug interactions via inhibition of drug metabolism and excretion in the liver. One of the principal steps in biliary excretion of xenobiotics is the active efflux from hepatocytes into bile canaliculi. Mrp2 is the major active transporter mediating this process for anionic substances. Therefore, the aim of this study was to investigate the effects of amiodarone treatment on the expression and function of Mrp2 transporter monitoring the kinetics of its endogenous substrate, conjugated bilirubin (CB).

Methods To determine the potential influence of amiodarone therapy on the kinetics of CB (supposing steady-state conditions), rats (n = 6) were treated for 4, 7 or 14 days either with amiodarone (25 mg/kg/day orally) or with vehiculum (phospate buffer saline) alone. During 120-minute kinetic study samples of bile, urine and plasma were collected and from the determined concentrations of CB (using Cobas Integra® 800, Roche Diagnostics) main kinetic parameters were calculated. Mrp2 expression was evaluated by Western blot analysis in total hepatic membrane fractions obtained from the rats after termination of the kinetic study.

Results The influence of amiodarone administration after 4-, 7- and 14-day pretreatment on renal and biliary clearance of CB is shown in Figure 1A and 1B. Amiodarone pretreatment increased the renal clearance along with duration of the treatment, reaching the highest value on day 14. The biliary clearance followed an inverse pattern, decreasing along with the treatment duration. The expression of

Mrp2 protein changed differently in the liver and kidneys. While in the kidneys it was increased during the whole 14-day period of treatment by 234–270% compared to controls, in the liver it firstly increased after 4 days to 117%, and later decreased to 82% of the control values (Figure 1C, D).

Discussion and Conclusion The biliary excretion of CB is mediated almost selectively by Mrp2 transporter. In kidneys, the major pathway is glomerular filtration with some part accomplished also by active transport, both basolateral uptake and canalicular efflux in proximal tubules. The observed changes in CB excretion may be partially explained by the modified expression of Mrp2 protein, which was down-regulated in the liver and up-regulated in kidneys. In conclusion, amiodarone treatment produced a shift in the excretion of CB from the hepatic to the renal pathway via altering the expression of the Mrp2 transporter.

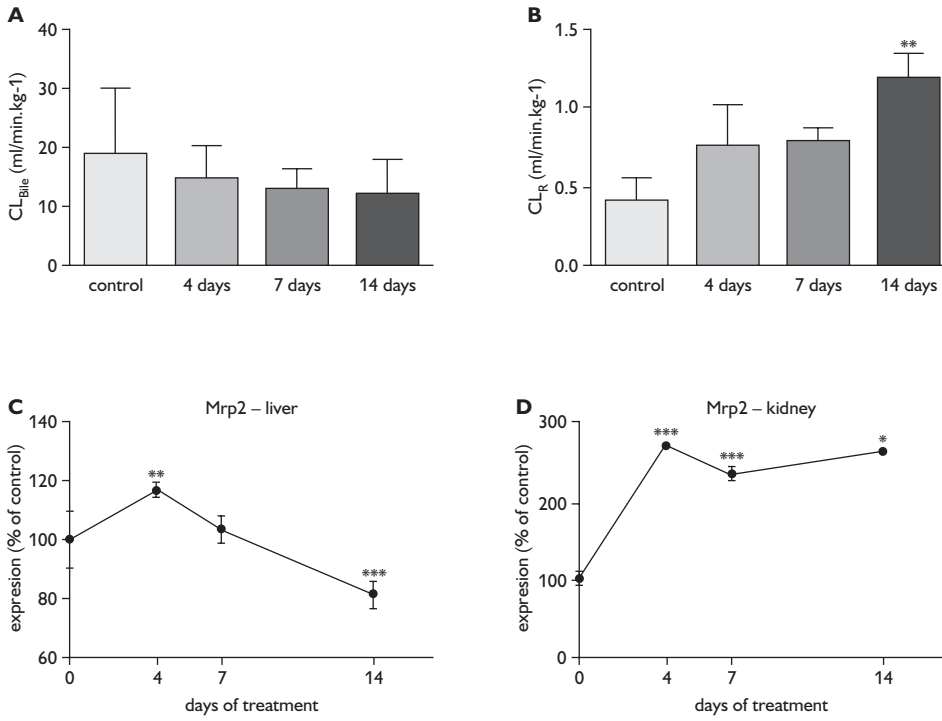

Figure 1 – Biliary (CL_{Bile}; A) and renal (CL_R; B) clearance of CB in amiodarone (4, 7, and 14 days) pretreated rats. Protein expression of Mrp2, in livers (C) and kidneys (D) after 4-, 7-, and 14-day amiodarone pretreatment determined using Western blot.
*Bar charts present means ± SEM (n = 6). Expression (mean ± SEM; n = 6) is presented as a relative optical density compared to the control animals; *, **, *** significantly different from control group on p<0.5; p<0.01; p<0.001, respectively*

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Wortmannin, a PI3 Kinase Inhibitor, Aggravates Myocardial Ischaemia-reperfusion Injury in Diabetic, but Not in Diabetic-hypercholesterolaemic Rats

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Key words: wortmannin – PI3/Akt kinase – eNOS, diabetes – hypercholesterolaemia

This project was supported by the grant number VEGA 1/4296/07.

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Introduction Activated PI3/Akt kinase pathway influences myocardial ischaemia-reperfusion injury (MIRI) by its antiapoptotic activity. One of the downstreams of Akt kinase is endothelial NO synthase which produces nitric oxide. In our previous in vitro experiment hypercholesterolaemia was found to abrogate an increased resistance of diabetic rat hearts to ischaemia-reperfusion injury. Aims of the study were to investigate the effect of wortmannin, a PI3 kinase inhibitor, on MIRI in diabetic and diabetic-hypercholesterolaemic rats and to determine the expression of eNOS and its allosteric inhibitor caveolin-1 in myocardial tissue.

Methods Male Wistar rats were divided into 3 groups – control (C), diabetic (DM) and diabetic-hypercholesterolaemic (DM-HCH). Experimental diabetes was induced by a single dose of streptozotocin (80 mg/kg, i.p.). Within 5 days, diabetic animals were fed by a fat cholesterol diet, which lead to hypercholesterolaemia. Plasma levels of glucose and total cholesterol and the amount of total cholesterol in the liver were measured to confirm the induction of diabetes and hypercholesterolaemia. *In vivo* MIRI was performed by occlusion of LAD coronary artery. After six minutes of ischaemia, the occlusion was loosened. During the subsequent reperfusion, ventricular arrhytmias were observed. Reperfusion arrhythmias were interpreted according to Lambeth Conventions and the arrhythmia score was set. Wortmannin was administered 15 minutes before the reperfusion (15 μg/kg, i.v.). The expression of eNOS and cav-1 in the left ventricular tissue was determined by SDS-PAGE and Western Blot and evaluated using densitometry.

Results and Conclusions Administration of a PI3 kinase inhibitor, wortmannin, to the control animals significantly increased the duration of ventricular fibrillation

(VF) (64.4s ± 48.7s vs. 11.2s ± 7.7s, $p<0.01$). In the diabetic rats a prolonged duration of ventricular tachycardia (VT) (141.4s ± 52.8s vs. 23.5s ± 7.3s, $p<0.05$) and ventricular fibrillation (VF) (58.7s ± 37.9s vs. 1.3s ± 0.7s, NS) was observed under the influence of wortmannin (Figure 1). The incidence of VT was increased by 20% and the incidence of VF by 100% in control group (Table 1). The incidence of VF in diabetic rats was increased by 60%. Both control and diabetic animals developed arrhythmias leading to a higher arrhythmia score (+31, +44% respectively). The mortality during reperfusion rose to 100% against 40% without the administration of wortmannin. On the other hand, in DM-HCH animals wortmannin decreased the incidence of VF by 20%. Only non-significant changes in duration of severe ventricular arrhythmias were observed. All animals of DM-HCH group survived the reperfusion phase. In the left ventricular myocardial tissue a trend to decreased eNOS expression was detected. The expression of caveolin-1, an allosteric inhibitor of eNOS, was not changed in any group.

Activation of eNOS depends not only on its expression, but also on its phosphorylation by Akt, a member of PI3/Akt kinase pathway. The trend to decreased eNOS expression in the myocardial left ventricle of DM-HCH rats might be the reason of different effect of wortmannin on in vivo MIRI in DM-HCH rats in comparison with diabetic and control group.

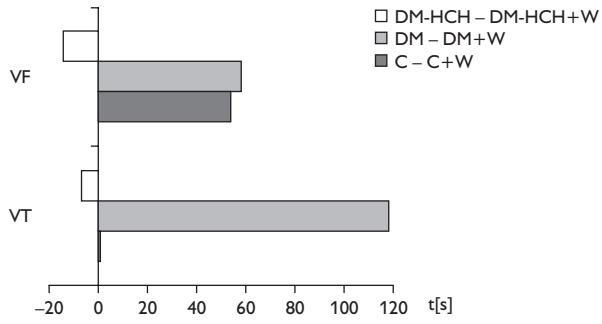

*Figure 1 – Changes in the duration of ventricular arrhythmias during reperfusion after the administration of wortmannin as compared to MIRI alone (**$p<0.01$ C vs. C+W, *$p<0.05$ DM vs. DM+W).*

Table 1 – Changes (Δ %) in the incidence of ventricular arrhythmias during reperfusion and in the arrhythmia score after the administration of wortmannin (W+) as compared to MIRI alone (W–)

	n		VT	VF	arrhythmia
group	W–	W+	(Δ %)	(Δ %)	score (Δ %)
control	6	5	20	100	31
DM	6	5	0	60	44
DM-HCH	8	10	0	–20	–1

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Toxicological Aspects of Chemopreventive Compounds

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Key words: Chemoprevention – Flavonoids – Cytochrome P450 – Carcinogenesis

The financial support from grants GA ČR 303/06/0928 and 203/06/0329, and the grant MSM ČR 0021620808 are highly acknowledged.

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Introduction Increasing attention is being paid to the possibility of applying chemopreventive agents to reduce cancer risk in humans. Among chemopreventive compounds phytochemicals are frequently used, as their intake is widely acceptable and considered to be safe because of their plant origin. Although multiple mechanisms are likely to be involved in the protective effects and the synergism of phytochemicals may count for the final beneficial effect, possible chemopreventive agents have been suggested: vitamin derivatives, phenolic and flavonoid agents, organic sulphur compounds, isothiocyanates, curcumins, fatty acids and d-limonene. These compounds, however, have to be considered as foreign compounds, which besides expected beneficial effects might exert negative activities. Possible adverse effects arise from the induction of cytochromes P450 (CYP) by chemopreventive compounds. These biotransformation enzymes are frequently involved in the activation of numerous carcinogens. While chemopreventive compound, e.g. flavonoid, effectively inhibits CYP mediated activation of particular carcinogen when the carcinogen and flavonoid are present simultaneously, the sequential intake of these compounds (carcinogen after flavonoid) might enhance the carcinogen activation via elevated CYP activities as a result of the enzyme induction by the flavonoid ingested formerly.

Aim of the study To approve dietary supplement to be a human chemopreventive agent, the mechanism of its action towards the desired target as well as all other interactions within the body should be known in details. The aim of this study was to examine interaction of selected chemoprotective agents with CYPs, namely their impact on the CYP induction.

Materials and Methods Compounds tested in this study were administered p.o. 60 mg kg^{-1} body weight to male Wistar rats (150 g) for 5 consecutive days. Microsomal fractions were prepared from rat tissues by differential centrifugation. The expression of CYP1A1/2 and 2B1/2 in liver and small intestine was determined

by Western blotting using CYP-specific antibodies. Inducers of CYP1A1/2 and CYP2B1/2, β-naphthoflavone and phenobarbital, respectively, were used as positive controls.

Results and Discussion The effect of selected compounds on CYP1A1/2 and CYP2B1/2 protein expression in rat liver and small intestine is shown in Figure 1. Comparing the effect on CYP1A and CYP2B, flavone and diallyl sulphide are potent inducers of both CYP familes. Of others, only flavanone is active in induction of CYP2B2. Similarly to liver, β-naphthoflavone and diallyl sulphide proved to be potent inducers of CYP1A1 in small intestine. Marked induction of CYP1A1 was observed also after curcumin, morin and rutin rat treatment. All compounds inducing CYP1A1 were active in CYP2B1 induction as well. Interestingly, flavonoids, flavon, flavanone and morine, exhibited differential induction in examined tissues. As namely CYPs of 1A family are involved in pro-carcinogen activation, the intake of the food supplements inducing these enzymes should be considered as a cancer risk factor.

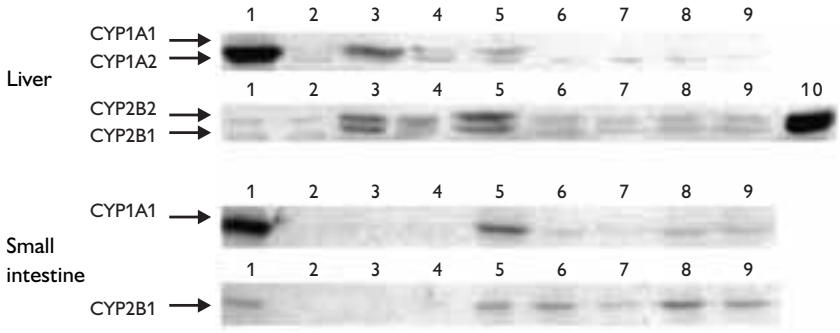

Figure 1 – Immunodetection of CYP1A1/2 and CYP2B1/2 in rat hepatic and intestine microsomes after pretreatment animals with β-naphthoflavone (1); control (2); flavone (3); flavanone (4); diallyl sulphide (5); curcumin (6); biochanin A (7); morin (8); rutin (9); phenobarbital (10).

Adipocytes as Model for Studying Drug Targets: the Example of PPARγ and Certain Post Receptor Signals

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Key words: Nitric oxide – Glitazones – Adipocytes – Resistin – Adiponectin

Supported by IGA MZ ČR NR/9379-3/2007, VZ MSM ČR 0021620807.

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Aims Adipocytes are suitable model for research work directed to obesity, metabolic syndrome.

They are used since 1974 as 3T3-L1 preadipocytes cell-lines or as isolated short-term cultivated subcutaneous and visceral cells. No longer they are considered as only fat-storing cells. Adipocytes secreted active molecules are continuously raising in number (about 30 molecules) including cytokines, low molecular weight signaling agents etc. The difference between different kinds of adipocytes-visceral, subcutaneous, perivascular, peritumorous adipocytes and cell lines opens the discussion of different results under various research conditions. PPARγ nuclear receptor is well known target of some antidiabetic drugs as glitazones predominantly situated in adipocytes. Glitazones induced PPARγ agonistic effect increases of adiponectin known to be a marker of insulin-sensitivity. Their adverse effect – weight gain is not so far elucidated as some authors believe to be just water retaining while other stress the adipocyte mass increase. New marker of insulin-resistance is believed to be resistin. Nitric oxide (NO) is well known as potent vasodilatator agent with many effects in different tissues icluding adipocytes. TNFα is a typical cytokine affecting many other signaling pathways including insulin-resistance. The aim of our work is to clarify some aspects of effect of PPARγ agonist, antagonist and β_3 agonist (strong lipolytic agent) / antagonist on adiponectin, resistin, TNFα and NO production in rat epididymal isolated adipocytes.

Methods After sacrifice of rats, the epididymal fat tissue was extirpated and chopped into small pieces. The chopped tissue was incubated in KRB buffer for 1 hour with collagenase (2 mg/2 g fat tissue). The isolated adipocytes were then pushed through nylon mesh with 500-μm diameter pores and rinsed 3 times with KRP buffer. Homogenized adipocytes were incubated in Petri dish kept in temperature of 37°C; in the mixture of 95% air and 5% CO_2 in Dulbecco modified Eagle's media with gentamicin and penicillin.

Different agents were added: Troglitazone (PPARγ agonist), SR-202 (PPARγ selective antagonist), BRL-27344 (β_3 selective agonist), SR 59230A (selective β_3 antagonist). After 12, 24 and 48 hours the levels of NO (NO_2-oxidative products) determined by Griess reagent, glycerol- colorimetric detection, adiponectin, resistin and TNFα were measured via ELISA kit methods.

Results Lipolysis was decreased after glitazone and SR-202 and the β_3 agonist-induced lipolysis (BRL-37344) was blocked by glitazone application. Resistin and adiponectin reacted as expected to glitazone addition (adiponectin increased, resistin decreased) after more than 24 h incubation. However, adiponectin level was paradoxically decreased after 24 hours but increased more than control after

48 hours. Moreover, BRL-37344 produced remarkable increase of resistin being attenuated by glitazone. NO exhibited increasing trend after troglitazone/SR-202 alone and in combination with BRL-37344. The BRL-37344 effect on NO being partially attenuated by addition of glitazone. TNFα showed no reaction on different agents alone or in combinations.

Conclusions Targeting PPARγ and NO release has a potential of development of new antidiabetic or antiobesity molecule. More work in evaluation of these molecules effects and signaling pathways is needed.

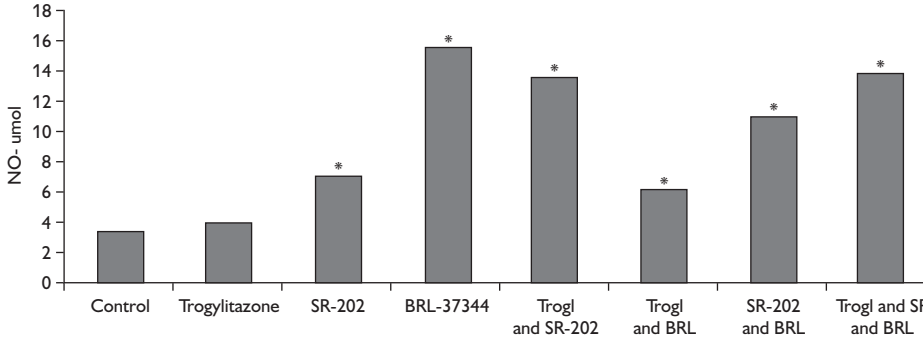

Figure 1 – The effect of Troglizone, BRL-37344 and SR-202 on NO production in isolated adipocytes
** statistically signif. values are marked*

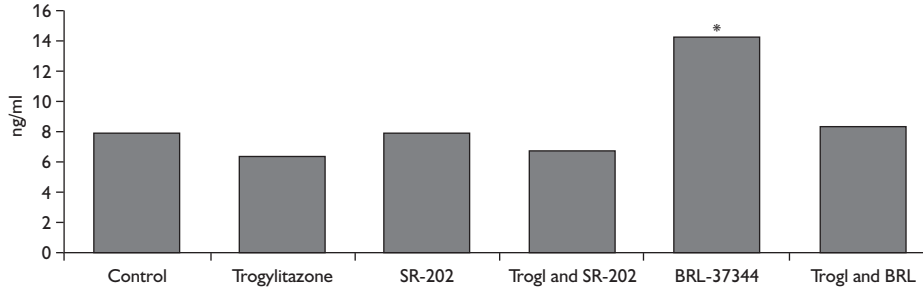

*Figure 2 – Resistin levels after 72 hours; * statistically signif. values are marked*

The Effect of Curcumin, Resveratrol and Selenium on the Activity of Selenoenzymes – Interaction with Cadmium

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Key words: Cadmium – Curcumin – Resveratrol – Selenium – Selenoenzymes

Supported by the Grant MSM ČR No. 0021620819 and by the Specific Research of Charles University in Prague – Faculty of Medicine in Pilsen.

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Introduction Resveratrol (RSV) is a naturally occuring polyphenol, studied mainly for its antioxidant and anticarcinogenic properties. Curcumin (CUR) – a natural phenolic compound, the major component of Curcuma longa, is considered to be a potent cancer chemopreventive agent with antioxidant effects. Selenium (Se) is an essential element which forms the active center of glutathione peroxidase and thioredoxin reductase – enzymes belonging to antioxidant defense system in the organism. Cadmium (Cd) is a toxic carcinogenic metal of great environmental and human health concern.

Aim To determine the effect of cadmium and natural antioxidants curcumin, resveratrol and selenium on the activity of selenoenzymes – glutathione peroxidase and thioredoxin reductase in rats.

Methods Adult male Wistar rats weighing 140–150 g were randomly assigned to 7 groups of 8/9 animals each: I – control, II – Cd, III – Cd+CUR, IV – Cd+RSV, V – Cd+Se, VI – CUR, VII – RSV. Curcumin (50 mg/kg b.w.) and resveratrol (10 mg/kg b.w.) were administered orally at 48h, 24h and 1h before cadmium administration (4 mg $CdCl_2.2.5H_2O$/kg b.w., ip). Selenium was administered as sodium selenite at a single dose equimolar to cadmium (*sc*, 15 min before Cd). At 24th hours after Cd administration, the animals were sacrified by decapitation. The level of reduced glutathione (GSH), lipid peroxidation (LP) and the activity of glutathione peroxidase (GPx) were estimated in liver homogenates as described previously The activity of cytosolic thioredoxin reductase (TrxR) was determined using commercial kit (Sigma).

In *in vitro* experiments, 1 mM concentration of antioxidants was used for the evaluation of effects on TrxR activity.

Results are expressed as mean ± SD, statistical evaluation was done by unpaired Student's t-test.

Results A. *In vivo* experiment: The results from experiment are summarized in Table 1. Twenty-four hours after Cd administration, a significant increase in TrxR activity and level of LP were found in liver homogenates compared to control group. The level of LP was decreased after pretreatment with curcumin and selenium. After Se + Cd administration, an increase in TrxR activity and GSH level and a decrease in GPx activity was found compared to Cd only treated group.

B. *In vitro* experiment: The activity of TrxR was not affected by resveratrol, inhibited by curcumin, and increased by selenium (190%, $p<0.01$)

Table 1 – Summary of results from *in vivo* experiment

	LP [nmol/g]	GSH [mmol/g]	GPx [mmol/g/min]	TrxR [U/mg of prot]
control	39.4 ± 7.3	4.11 ± 0.26	30.1 ± 2.2	1.36 ± 0.20
Cd	52.5 ± 4.8**	4.30 ± 0.40	29.6 ± 3.1	2.55 ± 0.21**
Cd + CUR	47.9 ± 3.5#	4.47 ± 0.39	30.0 ± 1.9	2.61 ± 0.33
Cd + RSV	50.1 ± 3.4	4.52 ± 0.61	29.6 ± 1.7	2.38 ± 0.32
Cd + Se	47.5 ± 4.1#	6.61 ± 0.40##	26.0 ± 2.7#	3.00 ± 0.39##
CUR	41.3 ± 7.4	4.67 ± 0.26**	28.2 ± 1.1*	1.38 ± 0.15
RSV	39.6 ± 3.0	4.84 ± 0.48**	27.5 ± 1.0**	1.30 ± 0.16

*p<0.05, **p<0.01 vs control, #p<0.05, ##p<0.01 vs Cd

Conclusions In the present study, the antioxidant effects of curcumin and selenium were demonstrated in Cd-induced oxidative liver damage in mice. The mechanism of antioxidant effect of selenium and curcumin has a different background, as can be deduced from experiment *in vitro*. The increase of TrxR activity caused by cadmium and potentiated by selenium are important findings deserving further study.

Metabolomics – The Keystone of a New Drug Discovery and Development Paradigm

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Key words: Metabolomics – Drug discovery – Drug development

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Introduction In the recent past, understanding structure-activity relationships in highly sophisticated but fairly "reductionist" models was accepted as decisive for the design of successful drugs: Molecular biology approaches continue to identify molecular targets thought to be relevant in disease modification, while combinatorial chemistry and biotechnology work in concert to provide the tools for the synthesis of vast numbers of compounds, and highly sophisticated- but rarely very complex – models to assess drug effects. However, along with these developments, drug R&D costs have dramatically risen in the last two decades, partly due to the immense expenditure involved in synthesizing vast numbers of compounds to be put through ever expanding screening programs designed to be

predictive of clinical drug kinetics, metabolism, toxicity and efficacy. Despite this enormous investment in preclinical selection and profiling, late stage toxicity and efficacy issues increasingly hamper successful drug development, and 20% to 40% of investigational drugs are discontinued due to toxicity concerns. Of the precious few compounds that remain to qualify for clinical studies, many go on to fail in pivotal human trials due to lack of efficacy or delayed toxicity.

Current challenges of drug R&D thus span the entire spectrum of life science research, from correct disease diagnosis over a detailed knowledge of disease mechanisms, to early and precise assessments of beneficial over adverse drug effects.

How does metabolomics fit into this scenario? Is it just another much hyped "omics" technique requiring huge technological investments and generating additional enormous graveyards of irrelevant data that no-one has the time to look at in detail? That the necessary technology is very expensive, creates vast amounts of data, and in particular, that this data is of dubious relevance are the main reasons preventing the integration of metabolomics into drug R&D.

To address the issue of relevance, it is useful to extend the central dogma of molecular biology to the sequence "DNA makes RNA makes Proteins/Catalysts makes small molecules", showing a multitude of small, non-protein molecules to be the final functional result of a large number of gene activation processes. An assessment of the complete "metabolome" of a living system thus represents the functional result of a very large proportion of the interplay of previous genomic and proteomic processes, and supplies supremely relevant biological endpoint readouts.

That small molecules are a very highly relevant endpoint, for example in human (drug) metabolism and toxicity, may be illustrated by the fact that the only regulatory acceptable in vitro cytochrome P450 induction data are measured metabolite quantities formed through the activity of cytochrome P450 isoforms in human hepatocytes. Unlike data on reporter gene activation, mRNA or protein expression, this "chemical" data represents the true functional state of the system under investigation. While this model is of course at best an extremely focussed "mini-metabolomics" assessment, it follows the very same logic and almost completely eliminates the danger of false positive or false negative results seen in gene activation or protein transcription data that very often do not translate into changes of enzyme activity and cell function.

While metabolomics is certainly challenging in terms of sample analysis, recent developments in chromatography and mass spectrometry have shown that the problems posed by the necessity to separate and detect many thousands of small molecules in one sample and to evaluate the resulting large data sets are on the way to being overcome. New separation and detection techniques such as UPLC-QTOFMS provide highly informative data and are more amenable to industrial R&D strategies than NMR-based metabolomics analysis. Similarly, the

evaluation of metabolomics studies, while impossible without the use of sophisticated data analysis due to the number of data points generated, can be achieved using novel approaches that are already firmly established in other areas of research where very large data matrices need to be handled.

Conclusion In conclusion, the ability to assess the results of enzyme activity at a functional level in health and disease through metabolomic techniques will improve our understanding of disease processes, and allow more accurate diagnoses through the identification of relevant biomarkers. Pharmacological intervention, rather than being based on the assumed modification of isolated receptor interactions, can be targeted and operate at the level of overall cellular/organism effects, exploiting the advantages of operating downstream of genome and proteome. The ability to monitor cellular state as a function of small molecule "effector" content provides a highly sensitive model supplying high content data for the early assessment of drug-induced adverse effects, as well as the identification of biomarkers relevant to toxicology and efficacy.

The immense power of metabolomics studies to provide high-content data supporting all areas of drug research and development should ensure that the technique is widely adopted in the pharmaceutical industry, despite the remaining technical and computational challenges.

Is the Pain Treated Adequately and Safely in Slovakia?

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Key words: Analgesics – Risk – Consumption

This work was supported by grant No. UK/94/2008.

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Introduction A progress in developing new therapeutical strategies in order to minimize risk of adverse drug reactions (ADRs) (new galenic forms, combination of non-steroidal antiinflamatory drugs (NSAIDs) with antiulcer drugs or misoprostol) was done recently followed with evaluation of analgesic drug – consequent withdrawals of molecules at high risk of health damage (e.g. phenacetine, aminophenazone, analgesic combinations containing barbiturates, rofecoxib).

Question about safety of drugs remains unanswered. The key role in the evaluation process plays drug risk perception. Pharmaceutical industry and control

members tried to improve safety and reevaluated the safety drug potential. Nevertheless they do not solve the the problem of optimal pharmacotherapy.

Critical situation in the treatment of pain lead World Health Organisation to design standardised treatment options, known as analgesic ladder. It is based on most distinguished characteristics of pain in practice – intensity. Important place in analgesic therapy have co-analgesics, drugs used from different reasons, which have secondary analgesic effect. These drugs include for example corticoids, antidepresants, anxiolytics, myorelaxants, anticonvulsive drugs, neuroleptics, anaestetics and H1 antihistamines.

Methods We collected annual wholesale data and drug safety reports from the State Institute for Drug Control in Slovak Republic during years 1998–2004. The analysis of consumption was based on ATC/DDD methodology.

Results Analysis of Slovak consumption showed that most used analgesic drugs were ibuprofen, diclofenac and paracetamol similarly as tramadol and fentanyl (from opioid analgesic group).

Data from State Institute for Drug Control in SR concerning spontaneus reports of ADRs showed, that analgesics belonged to the most reported drugs. Particularly ketoprofen in all observed years (15 reports in the year 1998, 11 reports in the years 1999 a 2000, 18 reports in the years 2001, 2002 and 2003 and 19 reports in the year 2004), in some years other analgesics – diclofenac (16 reports in the years 1999 and 2000), ibuprofen (10 reports in the year 1998 and 12 reports in the year 2002), metamizol (2 reports in the year 2001 a 7 reports in the year 2004) and paracetamol/acetaminophen (10 reports in the years 1998, 1999 a 2003 a 8 reports in the year 2004). Especially organotoxic ADRs were reported rarely.

Majority of reports were related to administration of nonopioid analgesics and only minority to opioids (only 1 report after morphine in the year 2000, other reports were connected with administration of tramadol).

Conclusion Used analgesics have their own ramifications. For example, opioids have no effect in neuropathic pain, non-steroidal anti-inflammatory drugs are weaker analgesics. We must allow risk factors and different characteristics, when choosing right drug. There is a danger caused due to an OTC preparations, commonly used in self-treatment.

The spectrum of analgesics used in medical practice is influenced by long-term involvement of national commitees for drug safety in developed countries (scandinavian countries, USA, Great Britain, France, Australia).

The number and quality of spontaneusly reported ADRs indicates insufficient interest of medical professionals in participating at this form of pharmacovigilance activity in SR despite of obligation to report observed ADRs by law. Number of ADR reports related to opioid analgesics was very small. One of the reasons may

be that doctors report at first visible adverse reactions. We found out skin disorders to be the most reported ADRs. Gastrointestinal damage and analgesics are generally connected together. Number of spontaneus reports in comparison with their usage did not reflect in adequately risk perception.

Our study showed that risk/benefit ratio should suggest relative safety of these drugs, but published data brought conclusive evidence about possible risk.

From Drug Metabolism to Drug Metabolomics in Mice

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Key words: Metabolomics – Metabolite identification – Ultra-performance liquid chromatography – Time-of –flight mass spectrometry – Gene knockout mice – Humanized mice

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Introduction The pharmacological action of drugs and the toxicological effects of chemical agents depend both qualitatively and quantitatively upon host metabolism. Without a detailed understanding of the metabolism of xenobiotics we would not fully comprehend the mode of action of many toxic agents, such as insecticides, chemical carcinogens, and cancer chemotherapeutic drugs, nor the pharmacokinetics and pharmacogenetics of many of today's widely used medicines. Historically, metabolite identification has been a slow and laborious process, often requiring synthesis of radiolabelled substrates. Drug metabolism has a history of adopting separation technologies as they emerge, for example, paper chromatography, TLC, GLC, and HPLC. The development of ultra-performance liquid chromatography (UPLC) by Waters Corporation in 2004 provided the drug metabolism field with the opportunity to engage in high-throughput (9 min per chromatogram) and high-resolution drug and metabolite separation. Coupled with quadrupole-orthogonal time-of-flight mass spectrometry (QTOFMS), which yields accurate masses of analytes in both positive and negative ion mode, UPLC offers a unique window into both *in vitro* and *in vivo* drug metabolism, since metabolites can be separated by UPLC and identified from their accurate masses and fragmentation patterns using tandem mass spectrometry (MS/MS).

A typical urine analysis by UPLC-QTOFMS yields approximately 5,000 positive and 5,000 negative ions. Performed in a series of animals such as mice, this generates huge and unmanageable data matrices of animal number, peak intensity,

retention time and m/z (mass/charge ratio) value. A matrix of 500,000 data points cannot be handled manually. Although pioneered within the field of nuclear magnetic resonance, where the data matrices are considerably smaller, metabolomics (some refer to this as "metabonomics") is a combination of high-end analytical chemistry combined with multivariate data analysis or, for example, machine learning algorithms. Metabolomics seeks to determine the concentration and flux of all small molecules in a biological matrix, tissue or organism and is particularly well suited to the comparison of responses between wild-type and transgenic mouse strains. When urines from control and drug treated mice are subjected to metabolomic analysis, it is relatively straightforward to identify drug metabolites. These may be subjected to MS/MS for structural identification. The use of software such as MarkerLynx®, MetaboLynx® and random forests machine learning algorithm aids in the detection and identification of drug metabolites.

When a metabolomic investigation of a drug is conducted in gene knockout or humanized mice, an evaluation can be made of the overall contribution of individual metabolizing enzymes and/or nuclear receptors to the metabolic map. Such published studies include aminoflavone, arecoline alkaloids, PhIP, melatonin and paracetamol. This same approach has also be employed to uncover biomarkers for nuclear receptor activation and for disease states.

Conclusions Metabolomics is in the process of transforming how drug metabolism studies are conducted. We may anticipate an explosion of this field, including into clinical pharmacology and diagnostics.

Pycnogenol Does not Influence the Levels of TBARs and NAGA in Mild Type Diabetes in Rats

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Key words: Diabetes – Pycnogenol – TBARs – NAGA

This project was supported by the grant VEGA SR 2/5129/25 and UK 283/2007.

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Introduction Diabetes mellitus is a metabolic disorder characterized with hyperglycemia and insufficiency of secretion or effect of endogenous insulin. Hyperglycemia, increases the levels of reactive oxygen and nitrogen species. These reactive compounds can cause several diabetic complications by triggering of different pathways such as glycation of proteins, autooxidation of glucose, polyol pathway and lipid peroxidation. One of the products of lipid peroxidation is malondialdehyde and its levels are increased in diabetic patients. Malondialdehyde can be assessed by the measurement of thiobarbituric acid reactive substances (TBARs). One other mechanism resulting in cell injury as a result of oxidative stress is the increase of the lysosomal enzymes activity. This activity can be determined by measuring of the levels of N-acetyl-β-D-glucosaminidase (NAGA). The aim of this work was to determine whether antioxidant Pycnogenol® can be useful in the lowering of oxidative stress in diabetes by measuring of the concentration of TBARs and NAGA in heart, kidney and liver in streptozotocin induced diabetic rats.

Methods In this experiment there were used male Wistar rats (300–350 g). They were divided in to three groups (n = 8 animals in each group): control group, diabetic group, in which diabetes was induced after the administration of streptozotocin 25 mg/kg i.p. three days sequential. In the third group were also diabetic animals; however, 14 days after third administration of streptozotocin, began the treatment with Pycnogenol® 50 mg/kg/day for eight weeks. After eight week of treatment the levels of preprandial and postprandial glycaemia were measured. The animals were sacrified and their hearts, kidneys and livers were taken for a biochemical assay. The levels of TBARs and NAGA were measured according to standard method. The results were expressed as the mean ± standard error of the mean and evaluated by using of Student's *t*-test. P values < 0.05 were considered significant.

Results and Conclusions Table 1 shows the body weight and the weight ratio of kidney, liver and heart in control, diabetic and treated animals. In this table there are also shown the changes of pre- and postprandial glycemiae in animals. The levels of preprandial glycemia were elevated in diabetic animals compared to control group (30.79 ± 1.94 *vs.* 5.58 ± 0.25 mmol/l; $p<0.001$). Pycnogenol® treatment lowered this levels significantly (12.85 ± 1.19 *vs.* 30.79 ± 1.94 mmol/l; $p<0.001$). The postprandial serum glucose levels were in diabetic rats significantly increased (35.96 ± 1.45 *vs.* 8.90 ± 1.67 mmol/l) compared to control animals. Pycnogenol® treatment suppressed elevated levels (29.63 ± 2.52 mmol/l), however, not significantly. The levels of TBARs (Table 2) were in kidney of diabetic animals significantly increased in comparison to control group (1.28 ± 0.16 *vs.* 0.85 ± 0.11; $p<0.05$) Pycnogenol® decreased the TBARs levels not significantly. The levels of TBARs in heart were increased inchmeal; Pycnogenol® did not influence this rising tendency. The TBARs levels in heart were elevated in both,

diabetic and treated rats. In none of organs were significant differences in NAGA levels between healthy, diabetic and treated animals.

The effect of Pycnogenol® in the treatment of diabetes (lowering of serum glucose levels) is probably independent on its antioxidative properties.

Table 1 – Weight of animals and the levels of preprandial and postprandial glycemia

	Weight [g]		Weight ratio [mg/g]		
	Whole body		Liver/Body	Kidney/Body	Heart/Body
Group	Start	End			
Control	331.25 ± 10.43	404.38 ± 11.24	28.56 ± 1.31	3.10 ± 0.16	1.64 ± 0.25
Diabetes	320.63 ± 8.26	303.75 ± 27.84*	35.52 ± 1.85°°	4.80 ± 0.43°°	2.28 ± 0.10°
DP50	335.00 ± 9.40	287.50 ± 14.42*	33.79 ± 1.81°	5.12 ± 0.37°°	2.30 ± 0.07°
	Glycemia [mol/l]				
Group	Preprandial		Postprandial		
Control	5.58 ± 0.25		8.90 ± 1.67		
Diabetes	30.79 ± 1.94*		35.96 ± 1.45*		
DP50	12.85 ± 1.19*#		29.63 ± 2.52*		

° = $p < 0.05$ *vs.* control; °° = $p < 0.01$ *vs.* control; * = $p < 0.001$ *vs.* control; # = $p < 0.001$ *vs.* diabetic group; DP50 – diabetic animals treated with Pycnogenol® (50 mg/kg/day)

Table 2 – Biochemical assessement of TBARs and NAGA in diabetic animals

	TBARs [nmol/mg protein]		NAGA [ug 4-nitrophenol/min/mg protein]		
Group	Kidney	Heart	Kidney	Liver	Heart
Contro	0.85 ± 0.11	1.38 ± 0.15	9.89 ± 0.65	0.23 ± 0.02	9.24 ± 0.28
Diabetes	1.28 ± 0.16*	1.59 ± 0.17	10.82 ± 0.39	0.25 ± 0.03	9.85 ± 0.38
DP50	1.00 ± 0.13	1.73 ± 0.22	12.57 ± 1.04	0.21 ± 0.02	9.88 ± 0.39

* = $p < 0.05$ *vs.*control; DP50 – diabetic animals treated with 50 mg/kg/day Pycnogenol®; TBARs = thiobarbituric acid reactive substances; NAGA = N-acetyl-β-D-glucosaminidase

Efficacy of Provinol in Ovalbumine-induced Hyperreactivity of the Airways in Guinea-pigs

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Key words: Red wine polyphenolic compounds – Nitric oxide – Allergen-induced hyperreactivity of the airways in guinea pigs

This project was supported by the grants APVT 2005/13 MFN-05 and VEGA 1/3375/06.

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Introduction The prevalence of allergic diseases has been increased all over the world. It is caused of interaction between genetic factors and environmental ones such as dietary changes. If this is one of the most cases, an appropriate intake of foods or beverages with antiallergic activities can prevent the onset of allergic diseases and ameliorate allergic symptoms. Polyphenols have anti-inflammatory, anti-allergic, antioxidant properties in respiratory tract and can play the role during asthma treatment. The aim of this study was to investigate the short-lasting effect of Provinol on tracheal smooth muscle reactivity 14 days after sensitization of guinea pigs by ovalbumine in vivo and in vitro conditions. The role of nitric oxide (NO) in the bronchodilator effect of Provinol was also evaluated.

Methods During *in vivo* experiments the value of specific airways resistance as a parameter of smooth muscle reactivity in response to bronchoconstriction mediator histamine (10^{-6} mol.l^{-1}) was used. Above mentioned parameter was investigated in Provinol group 30 minutes and 5 hours after peroral administration itself. Participation of NO in effect of Provinol was examined during simultaneous addition non-selective NO synthase inhibitor L-NAME (N^{ω}-nitro-L-arginine methyl ester) 30 minutes and 5 hours after intraperitoneal administration itself. During in vitro experiments the amplitude of tracheal smooth muscle contraction as a tracheal smooth muscle reactivity parameter in response to bronchoconstriction mediators histamine (10^{-8}–10^{-3} mol.l^{-1}), acetylcholine (10^{-8}–10^{-3} mol.l^{-1}) and non-specific bronchoconstrictor ovalbumine

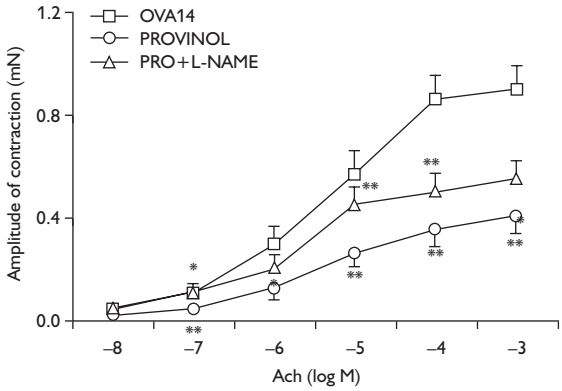

*Figure 1 – Amplitude of tracheal smooth muscle contraction in response to acetylcholine after 30 min pre-treatment with Provinol alone or in combination with L-NAME, in guinea pigs sensitized with ovalbumin. Data are mean ± SEM, n=12 for each group. *p<0.05, **p<0.01.*

(10^{-7}–10^{-3}g.ml^{-1}) was used. Bronchodilator effect of Provinol on isolated tracheal strips was examined after 30 minutes Provinol (10^{-4} mol.l^{-1}) alone, or after combination with L-NAME (10^{-6} mol.l^{-1}) pre-treatment.

Results and Conclusions In vivo conditions were found out that Provinol significantly reduced a value of specific airways resistance compared with controls 30 minutes after its administration. After 5 hours non-significantly decrease was happened. L-NAME was not led to significant fall of specific airways resistance. In vitro conditions Provinol caused decrease of contraction amplitude in response to specific bronchoconstrictors histamine and acetylcholine, whilst L-NAME partially inhibited this effect (Figure 1). After ovalbumine addition to organ baths similar changes were observed. After acute administration of Provinol the contraction of tracheal smooth muscle in guinea-pigs sensitization by allergen was inhibited. The influence over metabolism of nitric oxide played the role in acute bronchodilator effect of Provinol.

Phenotype Determination of CYP2D6 in Psychiatric Patients – Possibilities and Implications.

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Key words: Phenotype – CYP2D6 – Dextromethorphan – Psychiatry – Inhibition

This project was supported by a grant from MSM ČR No. 0021622404.

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Introduction Isoform 2D6 of cytochrome P450 (CYP2D6) is one of the most important enzymes involved in biotransformation of psychotropics in psychiatric patients. Many psychoactive drugs, mainly from the group of antidepressants (e.g. paroxetine, bupropion) and antipsychotics (e.g. risperidone, perphenazine) are potent inhibitors of CYP2D6. The final metabolic status of this isoform is greatly influenced by pharmacogenetic polymorphism as well as endogeneous and

exogenous factors (e.g. age, gender and xenobiotics-drugs). Thus, the determination of metabolic activity prior to the dose adjustment can be highly useful in some cases. The presentation will briefly summarize possibilities in CYP2D6 phenotype testing. In consequence, there was studied the influence of paroxetine on patient's metabolic status and phenotype conversion from extensive metabolizer (EM) to poor metabolizer (PM) status. Subsequently, we have studied the influence of CYP2D6 phenotype on clinical response in psychiatric patients.

Methods *Subjects:* The influence of paroxetine treatment on phenotype conversion from EM to PM – patients on paroxetine treatment ($n=55$). Influence of CYP2D6 phenotype on clinical response – patients with anxiety disorder on paroxetine treatment ($n=16$). Influence of phenotype on the adverse effects occurence (including sexual dysfunctions) in the long term paroxetine treatment – patients treated with paroxetine for more than 3 months ($n=50$). *O*-demethylation of dextromethorphan was used as a marker reaction for the determination of the metabolic activity of CYP2D6. After dextromethorphan administration, concentrations of dextromethorphan and dextrorphan in the urine were determined using HPLC assay. Antimode of 0.3 was used to distinguish PM and EM. Genotype determination of studied subjects is based on the detection of polymorphisms in CYP2D6 gene and on the assessment of the number of allele copies. The Hamilton Anxiety Scale (HAMA) was used for clinical response examination in anxiety disorder patients. The Arizona Sexual Experiences Scale (ASEX) was used for sexual dysfunction examination. UKU scale (Udvalg for Kliniske Undersøgelser) was used for examination of adverse effects of the paroxetine treatment.

Results and Conclusions The effect of paroxetine treatment on CYP2D6 phenotype is summarized in Table 1. Despite the "EM supposed" genotype in 34 patients, in 20 of them, PM phenotype was assessed. Influence of CYP2D6 phenotype on clinical response in depressive/anxiety patients is summarized in

Table 1 – Phenotype conversion in paroxetine treated patients

Genotype		No.	Phenotype EM < 0.3	Phenotype PM > 0.3
* 1 * 1	EM	34	14	20
* 1 * 4	IM	13	4	9
* 1 * 5	IM	3	0	3
* 1 * 3	IM	2	0	2
* 3 * 6	PM	2	0	2
* 3 * 4	PM	1	1	0
Totally		55	19	36

Figure 1. In general, earlier onset of the effect was observed in patients with poor metabolizer status. Influence of CYP2D6 genotype and phenotype on sexual dysfunction as a side effect of treatment: statistically significant increase in frequency of sexual dysfunction was observed in females with PM phenotype. We have observed no differences in adverse effects occurence in patients on long-term paroxetine treatment. In spite that the patients with PM phenotype react earlier on the treatment, we concluded that they are more vulnerable to the treatment of the substrates of CYP2D6 in general.

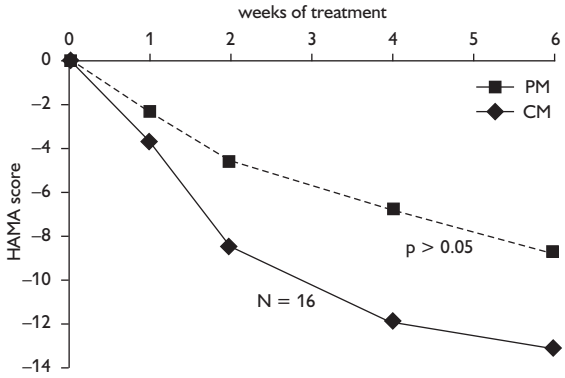

Figure 1 – Decrease of HAMA score in EM and PM depressive/anxiety patients on paroxetine treatment.

Substituted Pyridoindoles as Potential Antidiabetics: Effect on Glycolytic and Polyol Pathways in Intact Erythrocytes *in vitro*

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Key words: Pyridoindoles – Polyol pathway – Diabetic complications – Erythrocytes

This project was supported by the grant number VEGA 2/5005/26 and 2/0001/08, APVV 51-017905.

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Introduction The primary physiological function of the red blood cell is to transport oxygen. In addition, there are several metabolic functions that a red

blood cell must perform for its own survival. These include generation of metabolic energy (e.g. ATP), generation of reducing agents (e.g. NADH and NADPH), generation of 2,3-bisphosphoglycerate, and maintenance of ionic and concentration gradients across the cell membrane. They lack organelles, such as the nucleus and mitochondria; energy is generated exclusively by glycolysis. Since glucose is taken up by erythrocytes in an insulin-independent passive way, the excessive glucose under hyperglycemic conditions in diabetics is extensively metabolized also by an additional metabolic route, the polyol pathway, leading to the osmolyte sorbitol, produced by aldose reductase. Erythrocyte aldose reductase levels in diabetic patients were found to correlate well with severity of diabetic complications including cataract and retinopathy. Aldose reductase inhibitors fidarestat and epalrestat normalized the elevated sorbitol levels in erythrocytes of diabetic patients. Recently, novel carboxymethylated pyridoindoles, analogues of the efficient chain-breaking antioxidant stobadine, were designed, synthesized and characterized as prospective aldose reductase inhibitors endowed with antioxidant activity. Among the novel compounds developed (2-benzyl-2,3,4,5-tetrahydro-1H-pyrido[4,3-b]indole-8-yl)-acetic acid (1) and (2-phenethyl-2,3,4,5-tetrahydro-1H-pyrido[4,3-b]indole-8-yl)-acetic acid (2) were found the most efficient inhibitors of aldose reductase.

The aim of this work was to study the effects of the novel pyridoindole derivatives (1) and (2) on the complex metabolism of glucose in intact erythrocytes *in vitro*, including glycolysis and polyol pathway.

Methods Erythrocytes from Wistar male rats were incubated in shaking water bath at 37°C up to 24 hours (hematocrit 20%). The compounds studied were added to the erythrocyte suspensions in isotonic PBS to a final concentration of 100 μmol/l 30 min before adding 6 mmol/l glucose. The incubations were terminated after different time periods by cooling the suspensions in the ice bath followed by centrifugation at 3000 rpm for 15 min. In the supernatant, the concentration of glucose was determined with the BIO-LA-TEST Glucose GOD 1500 kit and lactate was determined spectrophotometrically on the basis of lactate dehydrogenase mediated NADH production. The concentration of sorbitol in the pellet of erythrocytes was determined by modified enzymatic analysis.

Results and Conclusions In a series of incubations of red blood cells with glucose, glucose consumption and lactate production were recorded as markers of glycolytic pathway, while sorbitol accumulation was determined as a marker of the polyol pathway (Figure 1). The effect of the compounds studied was evaluated and compared with stobadine and tetramethyleneglutaric acid standards. The effects observed were correlated with the uptake indices of the derivatives studied. Up to the concentration of 100 μmol/l, the compounds did not affect the plasmatic

membrane integrity of red blood cells, as determined by osmotic fragility measurements. The system of red blood cells incubated under physiological conditions with elevated levels of glucose was found a suitable cellular model to evaluate novel anti-diabetic agents under *in vitro* conditions.

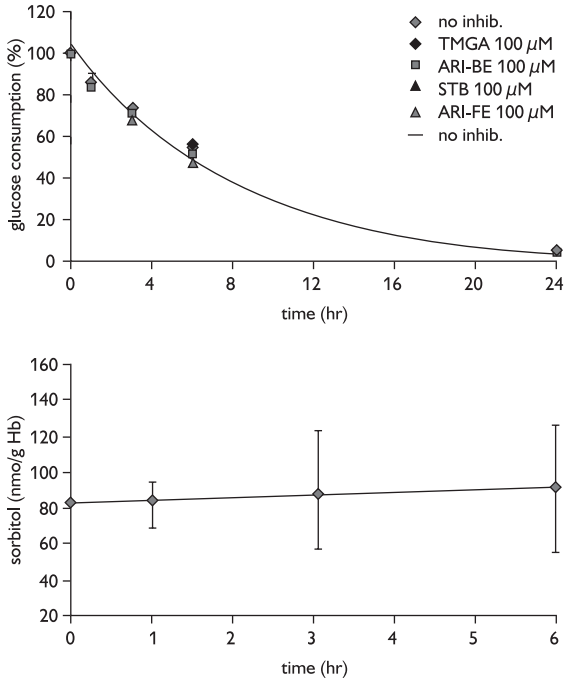

Figure 1 – Glucose consumption and sorbitol levels in red blood cells incubated with 6 mmol/l glucose.

Role of Ryanodine Receptor in Acute Phase of Anthracycline Induced Cardiomyopathy

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Key words: Ryanodine receptor – Anthracyclines – Cardiomyopathy – Calcium release

This project was supported by the grant of Slovak Society of Cardiology 2007 and UK/102/2008.

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Introduction Ryanodine receptor (RyR) – calcium release channel on the sarcoplasmic reticulum, plays critical role in regulation of calcium in cardiomyocytes. Use of anthracyclines in oncology is limited because of their cardiotoxicity. They have a biphasic effect on RyR – initially activate RyR, whereas after a few minutes, channel becomes irreversibly inhibited. We studied effect of short-term application of daunorubicine on RyR expression and tested its functional consequences.

Methods Wistar rats were treated for two weeks with daunorubicin (DAU, 3 mg/kg, i.p., in 48 h intervals), controls (CON) received vehicle. Left ventricular pressure (LVP), rate of contraction (dP/dtmax) and relaxation (dP/dtmin) were measured using left ventricular catheterization under Avertin anaesthesia. Cell shortening (at 0.5 Hz) and calcium sparks were measured in enzymatically isolated cardiomyocytes. Protein expression of RyR was detected using SDS PAGE and immunoblotting.

Results and Conclusions Application of daunorubicin led to a decreased function of the left ventricle (see table). On the other side, we observed increased cell shortening of isolated cardiac cells. Moreover, DAU cells showed increased incidence of resting spontaneous calcium release events ($p<0.05$).

Our data suggest that overexpression of RyR may be responsible for modulated calcium handling, which may contribute, at least in part, to the development of anthracycline induced cardiomyopathy.

Table 1 – Comparison of left ventricular function in control and daunorubicin-treated rats

	CON	DAU
n	7	8
LVP (mmHg)	132 ± 5	114 ± 6*
dP/dtmax (mmHg/s)	4976 ± 503	3179 ± 269*
dP/dtmin (mmHg/s)	–4144 ± 304	–2868 ± 297*
Cell shortening (%)	5.1 ± 0.4	6.7 ± 0.5*

*$p<0.05$

Effect of Antihypertensive Treatment on Pulse Pressure

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Key words: Hypertension – Pulse pressure – Ambulatory blood pressure monitoring

This project was supported by the grant of Slovak Society of Cardiology 2007.

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Introduction High pulse pressure (PP) was presented to be an appropriate parameter for the evaluation of chronic antihypertensive therapy. We tested whether such therapy alters PP and other blood pressure parameters in a different manner during 24-hour ambulatory blood pressure monitoring (24-h ABPM).

Methods Data from 24-h ABPM were compared between apparently healthy individuals (controls) and hypertensive subjects successfully treated (sBP/dBP 24-hour average below 130/80 mmHg) with antihypertensive medication in the absence (TH) or presence of additional risk factors such as diabetes mellitus and/or ischemic heart disease and/or myocardial infarction and/or over 60 years of age (TH+R). This groups were compared with non-successfully treated (sBP/dBP over 130/80 mmHg) hypertensives with absence (nTH) or presence of additional risk factors (nTH+R). We studied mean 24-hour systolic and diastolic blood pressure (sBP and dBP), mean arterial pressure (MAP) and pulse pressure (PP).

Results and Conclusions In TH and TH+R groups, sBP and dBP were appropriately controlled by antihypertensive medication, however PP was significantly increased in both (49±1 mmHg and 51±2 mmHg) as compared to the controls (43±1 mmHg; $p<0.05$). In nTH and nTH+R groups, values of sBP and PP were even significantly higher (60±2 mmHg and 76±3) as compared to controls as well as to successfully treated groups ($p<0.05$). PP was significantly positively correlated to sBP in controls and also in treated patients ($p<0.05$). Other parameters (age, body mass index, MAP, dBP) showed weaker or no significant relationship.

In conclusion, 24-h ABPM revealed significantly increased PP, in spite of controlled sBP and dBP. We propose that 24-h PP could provide an additional information in the evaluation of antihypertensive therapy, even though it depends on sBP.

Study of Metabolic Syndrome Using Tissue Cell Culture

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Key words: Tissue culture – Adipocytes – Macrophages – Adipose tissue – Metabolic syndrome

This project was supported by the grant IGA VFU No. 112/2008/FaF.

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Introduction Metabolic syndrome represented by the presence of obesity together with hyperinsulinemia associated with insulin resistance is the main mechanism in pathogenesis of cardiovascular diseases resulting in atherosclerosis. In recent studies, an important role of metabolic syndrome proteins (adipokines) in adipose tissue is pointed out. Cytokines within adipose tissue originate from adipocyte, preadipocyte and other cell types. Adipocytes secrete various adipokines to control the functions of other organs and cells. There is number of endocrinal products of adipocytes (adiponectin, A-FABP, resistin, leptin, TNF-α and others), which could be of clinical value for metabolic syndrome development. Expression of proinflammatory cytokines including TNF-α, IL-1, and IL-6 is increased in adipose tissue in the obese state, due to its secretion by macrophages. The use of tissue cell culture in various diseases research is more frequent. Aim of our work is to establish and evaluate most convenient methods for assessment of various endogenous (adipokines) and exogenous (newly synthesized compounds) substances effects on metabolic syndrome proteins. Another goal is to establish convenient cell line for evaluation of the involvement of inflammation in metabolic syndrome development.

Methods In our work we used two cell lines for study of metabolic syndrome proteins. To evaluate the role of adipose tissue, immortalized mice embryo 3T3 L1 cell line growing in DMEM with 10% Foetal Bovine Serum (FBS), was used. To study inflammation impact on adipose tissue, human monocytic leukaemia THP-1 cell line growing in RPMI with 10% FBS, was used. The level of adiponectin in growth medium of 3T3 L1 preadipocytes was evaluated by ELISA and mRNA assessment on day 0, 3 and 6 of the experiment. In THP-1 cells, differentiation into macrophage-like cells using phorbol diester was induced. The level of selected proinflammatory proteins was measured using specific mRNA detection.

Results and Conclusions: Based on our results we can conclude that level of adiponectin secreted by 3T3 L1 preadipocytes in growth medium was extremely low, which indicates more important role of matured adipocytes in metabolic syndrome development. To obtain the most relevant systems for investigating obesity-related morbidities, the embryo preadipocytes and cultured adipocytes (3T3 L1 cell line) are one of the most suitable cell lines. THP-1 cell line may offer a useful model for the study of inflammation impact on adipose tissue and relation between proinflammatory cells and (pre)adipocytes. Despite of extensive scientific efforts, all mechanisms leading to the effect on adipose tissue metabolism and unambiguously proving causality with the development of the metabolic syndrome are not precisely known at the present.

SNP Associated with IBD – Genetical Basis of Disease Prevalence, Safety and Effectivity of Pharmacotheraphy

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Key words: IBD – SNP-polymorphism – TPMT – MTHFR – NOD2/CARD15

This work was supported by Grant IGA MZ ČR, No. NR 9342-3/2007.

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Introduction Crohn's disease (CD) and ulcerative colitis (UC) are clinical subtypes of the inflammatory bowel disease (IBD). Although the aetiology of these disorders are not fully understood, there is strong evidence to suggest that genesis of both CD and UC is dependent on genetic and environmental components. Several different genes associated with the IBD were identified in human genome studies. We have focused on the candidate genes most frequently mentioned in literature. We studied genes ICAM-1, NOD2/CARD15, CCR5 and their five polymorphisms. Nonstandard alleles of them are thought to be involved in disorders of the human immune system. Different types of genes have been studied in context of therapy (primarily treatment of immunosuppressive drugs e.g. azathioprine or methotrexate) because many of them have an important delayed side effects. We studied SNP's in gene for Thiopurine-S-methyltransferase (TPMT) and Methylentetrahydrofolatereductase (MTHFR), which influenced the pharmacokinetics of these two drugs.

The aim of this study was to find a relationship between presence of nonstandard alleles and IBD occurrence and between TPMT and MTHFR-genotypes and clinical output of pharmacotherapy.

Methods We used PCR and its modifications – PCR-REA, multiplex PCR and Real-Time PCR to determine allelic variants of these genes. For statistical evaluation of our data we used χ^2-test (Unistat 5.1).

*Genes polymorphisms vs. developement of IBD***:** We studied presence of the nonstandard allelic form in gene ICAM-1 (Lys469Glu), two SNP's (Arg702Trp, Gly908Arg) and one frameshift mutation (Leu1007fsinsC) in gene NOD2/CARD15 and 32 bp deletion mutation in gene for CCR5.

Genes polymorphisms vs. clinical manifestation of IBD: We analysed our results of genotyping to investigate influence of mutation of genes on kind of manifestation of disease. We observe the age of disease manifestation, anatomical localization and activity of inflammation. Data was taken from patient's medical documentation and questionary.

Genes polymorphisms vs. pharmacotherapeutical output: We focused on study of two SNP's of gene MTHFR (C677T and A1298C) and three SNP's of gene TPMT (G238C, G460A, A719G). We analysed occurence of the most important side effect of azathioprine treatement – leucopenia in connection with presence of nonstandard alleles in monitored genes.

Results and Discussion *Genes polymorphisms vs. developement of IBD:* Allelic forms are tested in group of 102 patients with CD, 35 patients UC and 77 healthy volunteers. Our results proved that SNP in gene ICAM-1 (Lys469Glu) is in strong statistical association with presence of CD ($p=0.0003$). SNP in gene NOD2/CARD15 (Arg702Trp) is more specific for patients with CD than UC ($p=0.0323$). Another mutation of NOD2/CARD15 gene (Leu1007fs insC) is also statistically associated with CD ($p=0.0193$). We confirm hypothesis that CD is more influenced by genes polymorphisms than UC. The CD patients have more SNP's in theirs genotype if compare with UC patients.

Genes polymorphisms vs. clinical manifestation of IBD: We dispose medical data of 51 CD patients and 22 patients with UC diagnosis. Patients with agressive form of disease (occurence of fistulas) has 32bp deletion in CCR5 gene more often that patients with mild disease. Mutation in CCR5 is also more frequent in younger UC patients. In other hand in no-fistula patients group are often ICAM-1 mutation (Lys469Glu). In group of CD patients ICAM-1 mutation is associated with earlier disease manifestation (before 16th year). Patients with extensive form of UC have less mutations in NOD2/CARD15 gene. If we mentioned behavioral factors, there are significance between smokers in CD group, compared with UC group. Active smoking is thought to be preventive factor of occurence of UC.

Genes polymorphisms vs. pharmacotherapeutical output: We studied TPMT and MTHFR SNP's in case of theirs influence to pharmacotheraphy with azathioprine and methotrexate. Serious leucopenia is statistically frequent in patients with SNP's in TPMT gene ($p<0.01$). We found no relationship between SNP's in MTHFR gene and manifestation of leucopenia in patients treated with azathioprine or methotrexate.

Atypical Antipsychotics and the Risk of Metabolic Syndrome

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Key words: Atypical antipsychitics – Metabolit syndrome – Weigh gain

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Introduction Metabolic syndrome as a complication of atypical antipsychotic therapy has become an extremely important issue. Studies on adverse metabolic effects of atypical antipsychotics have repeatedly described an increased incidence rate of individual components of the syndrome after the start of the therapy. This study investigated mainly the problems of weight regulation associated with abdominal obesity correlating with weight gain, and glucose metabolism and dyslipidemia. Adverse metabolic effects of atypical antipsychotics pose a problem with serious clinical consequences.

In order to ensure the safety, medication adherence and compliance of the therapy, a basic examination should be performed before the treatment is started. Such examination should identify predisposed individuals. Moreover, all patients should be actively monitored during the therapy with atypical antipsychotic drugs.

Methods A sample of 20 schizophrenic patients taking olanzapine was monitored. Ten of them were *drug naive*, while the other 10 had been on risperidone before the start of olanzapine treatment. No patient took drugs of any other groups that could have effect on weight gain. Using the administration of Excomet software that uses criteria defined by NCEP ATP III (National Cholesterol Education Program Adult Treatment Panel III) the incidence of risk factors of metabolic syndrome was investigated in all patients on their admission into hospital. Furthermore, weight gain dynamics was monitored during a four-week olanzapine therapy.

Results and Conclusions Risk factors of the development of cardiovascular diseases and the manifestation of diabetes mellitus type 2 were observed in 25% of patients. Weight gain was noted in all patients. During a four-week therapy, weight gain in *drug naive* patients was characterized by $p < 0.001$ and in *non drug naive* patients by $p < 0.01$.

Weight gain, the most frequent adverse effect of atypical antipsychotics, is closely related with the development of metabolic syndrome. Patients that show signs of weight gain during the therapy should be appropriately educated. At the same time, an effective weight regulation strategy should be adopted and appropriate measures taken. In the first stage, these measures include particularly cognitive and behavioral techniques to change eating habits, an adequate diet and increased physical activity. Where these measures are not effective enough or other adverse metabolic complications occur during the therapy, pharmacological treatment should be considered and the patient should be further monitored by a specialist.

Effect of Carvedilol on Trace Elements Levels and Antioxidant Status of the Liver – Interaction with Cadmium

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Key words: Carvedilol – Cadmium – Oxidative stress – Essential elements

This work was supported by the grant MSM ČR No. 0021620819 and the Specific Research of Charles University Faculty of Medicine in Pilsen.

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Introduction Carvedilol is a combined β_1-, β_2-, and α_1-adrenergic blocking agent approved for the use in cardiovascular pharmacotherapy. It has been also reported to posses antioxidant and metal chelating activity. Cadmium is carcinogenic metal with prooxidant activity.

Aim This study was undertaken to evaluate the effect of carvedilol on cadmium-induced oxidative damage and trace elements levels in the liver of mice.

Methods Male CD mice (Velaz Prague, CZ), 28 ± 2 g of b.w., were divided into 4 groups of 8/10 animals as follows: I. Control, II. Carvedilol, III. Cd, IV.

Cd+carvedilol. Carvedilol was administered orally (10 mg/kg b.w.) 48h, 24h and 1h before cadmium chloride injection (33 μmol/kg, ip.). After 24h, the lipid peroxidation (LP), reduced glutathione (GSH) level and the activity of antioxidant enzymes glutathione peroxidase (GSH-Px) and catalase (CAT) were estimated in liver homogenates. Cadmium and trace element concentration were determined in the liver tissue by AAS.

Statistical evaluation was done by ANOVA, followed by Tukey-Kramer test. Mean ± SD values are presented.

Results Carvedilol treatment did not affect the changes in trace elements levels induced by cadmium administration (Table 1). Though, the increase of hepatic Cu level in carvedilol-only treated mice corresponds to coordination behavior of carvedilol towards copper, found in *in vitro* experiments.

Table 1 – Trace element contents in the liver of cadmium and carvedilol treated mice [μg/g]

Group	Ca	Mg	Zn	Cu	Fe	Cd
Control	30.0 ± 3.0	228 ± 9	21.8 ± 1.5	3.38 ± 0.20	88 ± 33	<0.5
Carvedilol	30.9 ± 1.8	232 ± 10	23.2 ± 1.8	4.26 ± 0.24*	77 ± 17	<0.5
Cd	76.2 ± 24.3*	230 ± 29	33.6 ± 2.9*	4.67 ± 0.78*	119 ± 20*	21.4 ± 2.9*
Cd + carvedilol	78.9 ± 31.8	213 ± 34	30.9 ± 7.0	4.15 ± 0.63	114 ± 29	20.2 ± 4.0

Concentrations in μg/g of wet tissue; Significant differences: *p<0.01 vs. control group;

Carvedilol significantly decreased LP and prevented the decrease of CAT activity caused by cadmium administration (Table 2). Other parameters of antioxidative defense system remained unaffected by carvedilol pretreatment. A decrease in GSH-Px activity was found in carvedilol-only treated group.

Table 2 – Effects of cadmium and carvedilol treatment on oxidative state parameters

Treatment	LP (nmol MDA/g)	GSH (μmol/g)	GSH-Px (μmol/g/min)	Catalase (k/g)
Control	46.1 ± 4.9	7.68 ± 0.98	8.51 ± 1.72	36.6 ± 6.1
Carvedilol	52.1 ± 5.7	7.43 ± 0.34	6.67 ± 0.90*	39.2 ± 5.2
Cd	70.1 ± 3.5*	2.79 ± 0.66*	9.45 ± 1.73	29.2 ± 5.0*
Cd + carvedilol	53.8 ± 7.5#	2.66 ± 0.68	8.06 ± 1.45	37.7 ± 4.9#

Significant differences: *p<0.01 vs. control group; #p<0.01 vs. Cd-group

Conclusions The antioxidant effect of carvedilol in Cd-induced oxidative liver damage was proved. The concentration of cadmium and the changes in trace elements levels induced by cadmium were not influenced by carvedilol

pretreatment. Thus, the antioxidant effect of carvedilol is probably not based on the formation of metal-complexes with carvedilol.

Acknowledgement The authors are thankful to Zentiva, a.s. for the supply with carvedilol.

Comparative Study of Carvedilol and FoA3nDMFE in Improving Heart Function after Myocardial Infarction in Rats

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Key words: Isoproterenol – FoA3n DMFE – Carvedilol – Myocardial infarction

This project was supported by the grant number APVT-51-31104, VEGA 1/4244/07, 2/6079/26 and UK 155/2008 for PhD student (EK).

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Introduction Recent studies have shown the benefits of beta blocker therapy in many patients with heart failure. Carvedilol, the first beta blocker labeled for the treatment of heart failure, has been shown to improve the left ventricular ejection fraction. Because of the side effects of betablockers, the synthesis of new potential beta blockers is still topical.

The aim of this work was to compare the effect of new potential beta blocker FoA3n DMFE with carvedilol on heart function after myocardial infarction.

Methods Post infarcted remodeling in rats was induced by a single injection of isoproterenol (150 mg/kg sc, Iso 150, n=9). Animals were treated with carvedilol (10 mg/kg 13 days, sc, Iso+ Car, n=5) or "new drug" (ND) (1 mg/kg 13 days sc, Iso +ND, n= 5). Blood pressure was measured using the tail cuff plethysmography. The electrocardiograms were recorded in anesthetised animals (thiopenthal 45 mg/kg, ip) as well as isolated hearts according to the Langendorff.

Results and Conclusions Significant changes in blood pressure among observed groups were not found. After single high dose of Iso the dilatation was manifested

by thiner LW free wall (3.06 ± 0.16 vs 3.32 ± 0.52 mm) , decreased heart weight (1.18 ± 0.09 vs 1.22 vs 0.19 g) and left ventricle weight (LVW) (0.74 ± 0.07 vs 0.78 ± 0.13 g) in comparison to the control rats. The dilatation affected electrical activity of the hearts, too. In contrast to control rats QT interval was overextented and ectopic ventricular activity was increased in Iso150 animals. In premedicated animals with new potential beta blocker FoA3n DMFE we observed the increased of heart weight (1.24 ± 0.06 vs 1.14 ± 0.04 g) and LVW (0.96 ± 0.05 vs 0.82 ± 0.07 g) in comparison to the carvedilol rats. Both drugs shortened the QT interval and supressed ectopic ventricular activity. Our results show that carvedilol and FoA3n DMFE diminished emergence of heart remodelling induced by increased hemodynamic overload.

Sulodexide Exerts Protective Effects on the Endothelium in the Model of Streptozotocin-induced Diabetes in Rats

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Key words: Endothelial dysfunction – Sulodexide – Diabetes mellitus

This project was supported by the grant VEGA number 1/0314/08.

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Introduction Endothelial dysfunction induced by diabetes mellitus belongs to the factors increasing cardiovascular risk. Agents with supposed endothelium-protective properties as sulodexide may decrease vascular damage. Effect of sulodexide (glycosaminoglycan composed from heparansulphate and dermatansulphate fractions) on endothelial dysfunction in experimental model of diabetes mellitus was investigated.

Methods Diabetes was induced by streptozotocin (3 × 30 mg/kg, i.p., administered on 3 consecutive days) in Wistar rats. Animals were divided into 4 groups: controls, sulodexide treated (100 UI/kg/d), diabetes and diabetes treated with sulodexide. After 10 weeks of diabetes acetylcholine induced

endothelium-dependent relaxation of phenylephrine-precontracted mesenteric artery and aorta was evaluated. The number of circulating endothelial cells in blood was calculated in Buerker's chamber.

Results and Conclusions Endothelium-dependent relaxation to acetylcholine of vessels obtained from diabetic rats was impaired in mesenteric artery and aorta accompanied with significant increased endothelaemia: controls 1.875 (0.75; 2.5) vs. diabetes 3.875 (3.25; 5.75). Sulodexide treatment in diabetic rats after 10 weeks significantly improved relaxation to acetylcholine only in the mesenteric artery and significantly decreased the number of endothelial cells in blood: diabetes 3.875 (3.25; 5.75) vs. diabetes with sulodexide 2.25 (1.75; 3.5).

The results have shown that sulodexide improves endothelium-dependent relaxation to acetylcholine in small arteries and decreases endothelaemia as a marker of endothelial injury in experimental diabetes in rats.

Comparison of Different Procedures of Sample Processing in Relation to the Sensitivity of PCR Pathogen Detection

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Key words: Real-time PCR – Detection of pathogens – DNA isolation

This work was supported by the GA of the Academy of Sciences of the Czech Republic No. AX00310701, and from the research project of the State Office for Nuclear Safety No. SUJ 7056581301.

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Introduction Due to the need to use the early goal-directed pharmacotherapy, diagnostics of bacterial diseases based on PCR detection of DNA specific sections has been increasing recently. Lots of manufactures are currently offering commercial kits for DNA isolation. Practically all the manufactures speak highly of their products and no data are available to make it possible to compare the suitability of individual kits for individual applications.

Work with dangerous pathogens pose risk of infection to the laboratory staff. Therefore, it would be suitable to inactivate the samples prior to their processing and thus minimise risk for infection. Majority of the commercial kits, however,

does not fit to thermal treatment of samples. Therefore, the report deals, on the one hand, with modifications of isolation procedures and, on the other hand, it mainly monitors finding of the most efficient treatment of the samples for the detection of bacterial pathogens by means of real-time PCR after their thermal inactivation.

Material and Methods Vaccination strain of Bacillus anthracis was selected for the work as a model organism. Efficiency of isolation of bacterial DNA was determined by means of real time PCR using a commercial kit Realart B. anthracis RG PCR Kit and on the basis of quantity and cleanliness of isolated DNA determined by spectrometer. Three different kits of Qiagen for DNA isolation were compared: QIAamp DNA Mini Kit, QIAamp Ultrasens Virus Kit, QIAamp DNA stool Kit. They were tested for the influence of sonication and lysozyme digestion by boiling the inactivated on the isolation efficiency.

Results The following was detected: Boiling prior to isolation by selected kits according to the instructions will decrease the yield of DNA and sensitivity of PCR reaction. Boiling sample after adding of lysys buffer will not affect the yield of DNA or the sensitivity of PCR reaction. Selected dose of sonication of DNA will decrease the sensitivity of PCR reaction (DNA is most probably damaged). Sonication of the unboiled sample will increase the yield of DNA only insignificantly but the sensitivity of PCR reaction becomes decreased – DNA is damaged by sonication. Sonication of the sample boiled prior to the addition of lysys solution will decrease the sensitivity of PCR reaction – DNA is damaged by sonication. Sonication of the sample boiled in lysis solution will increase the yield of DNA, but is has no impact on the sensitivity of PCR reaction – a higher yield of DNA is

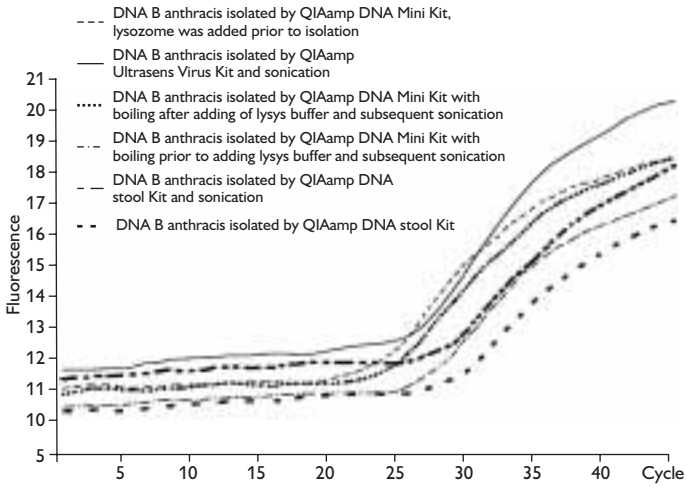

Figure 1 – Demonstration of testing the effect of individual modifications of DNA isolation on the sensitivity of detection of B. anthracis by means of real-time PCR after the samples processing by means of the selected kits.

compensated by its damage. However, it is not necessary to incubate the samples by lysozyme or proteinase K.

Comparable results of the sensitivity of PCR reaction were achieved with QIAamp DNA Mini Kit with the initial lysozyme digestion – standard procedure without thermal inactivation and with the samples boiled in lysis solution isolated by QIAamp Ultrasens Virus Kit using sonication and QIAamp DNA Mini Kit. When sonication was used in isolation by means of QIAamp DNA Mini Kit, incubation with neither lysozyme nor proteinase K had no impact on the sensitivity of PCR reaction.

Conclusion It follows from the results that effective isolation of DNA needs the addition of lysis solution to the sample prior to its boiling. Then incubation and proteinase K have no effect on the efficiency of isolation and boiling with subsequent sonication will replace even the lysozyme digestion.

The best appears the use of QIAamp DNA Mini Kit with sample boiling in the presence of detergent subsequently sonicated when incubation both by lysozyme and proteinase K will be omitted. Such procedure can not only substantially accelerate the isolation and simplify it but it also allows for the considerable increase of the laboratory staff safety.

Do the Female Hormons and Repeated Administration of Methamphetamine Affect the Spontaneous i.v. Self-administration of the Drug in Rats?

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Key words: Methamphetamine – Behavioural sensitization – Gender differences – i.v. self-administration – Rats

Supported by the research project from MSM ČR No. 0021622404 and by the Masaryk University Rector's grants No. 20061411C0002 and 20071411C0001.

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Introduction The female animals were already recorded to respond differently to methamphetamine (MET) abuse than males in clinical and preclinical experiments. These gender dissimilarities may be caused by the influence of estral cycles and different susceptibility to the behavioural sensitization phenomenon.

Methods The model of i.v. self-administration of MET (or saline as control) combined with fourteen days (14 D) of intermittent pretreatment with MET (or saline) was used hypothesizing potential influence of behavioral sensitization to MET on the spontaneous intake in twenty-one i.v. self-administration everyday sessions (120 min.) in male and female ovariectomized rats with and without estrogen substitution. Therefore two female groups were maintained in estrus-like conditions during the whole experiment (estradiol depot form 0.28 mg/kg i.m. once a week) while other two groups in anestrus (weekly application of saline i.m.). Adult male (M), female estrogenized (Festr) and female ovariectomized (Fovx) Wistar rats were randomly divided into four groups each with following treatment: n_1(M)= 6–14 D of pretreatment with saline (1 ml/kg/day i.p.), 14 D of wash-out period, twenty-one days of i.v. self-administration of saline; n_2(M)=12–14 D pretreatment with saline (1 ml/kg/day i.p.), 14 D wash-out period followed by twenty-one i.v. self-administration of MET sessions; n_3(M)=6–14 D pretreatment of MET (0.5 mg/kg/day i.p.), 14 D wash-out period, twenty-one i.v. self-administration of saline sessions; n_4(M)=12–14 D pretreatment of MET (0,5 mg/kg/day i.p.), 14 D wash-out period, twenty-one days of i.v. self-administration of MET. The same medication received the female groups n_1(Festr) – n_4(Festr) and n_1(Fovx) – n_4(Fovx). During the wash-out period the surgery and recovery required for i.v. self-administration was performed. Operant chambers produced by Coulbourn Instruments, USA, were used in a fixed ratio (FR) schedule of reinforcement to define the responses of the animals. For statistic analysis of drug intake the Mann-Whitney U test was applied.

Results and Conclusions There was no different saline self-administration in rat groups pretreated either with saline or MET. However (see Figure 1 and Table 1), significantly lower doses of MET were self-administered by rats pretreated with MET comparing to those pretreated with saline in all main groups (M, Festr, Fovx). The Festr groups self-administered significantly higher doses of MET than M groups with the same treatment did. Nevertheless the Fovx groups' intake was significantly lower than in M and Festr groups. All the animals experienced

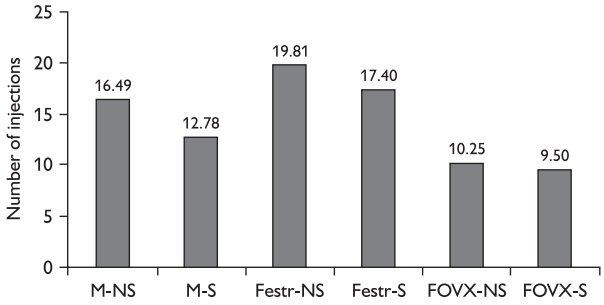

Figure 1 – This graph shows the mean values of MET intake during the whole experiment (the saline intake is not shown).

previously with effects of MET in the phase of pretreatment showed the lower self-administered intake of MET. This might indicate that MET produced more profound rewarding effects in rats previously sensitized to the drug as shown also in the case of amphetamine. In summary, the female groups in estrus-like conditions self-administered higher doses of MET than male groups with the same treatment however, the females in anestrus self-administered even lower doses than males. These results indicate certain enhancing effect of estrogens on MET abuse coinciding with previous studies and show a possible protecting effect of testosterone suggested in literature.

Table 1 – This table describes the statistically compared results of the experiment

Influence of behavioural sensitization		Influence of sex hormones in sensitized animals (S)		Influence of sex hormones in non-sensitized animals (NS)	
M	***	M : Festr	**	M : Festr	***
Festr	***	M : Fovx	***	M : Fovx	***
Fovx	n.s.	Festr : Fovx	***	Festr : Fovx	***

The Importance of the Hepatotoxicity Model: an Experience with D-galactosamine and Resveratrol

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Key words: Hepatotoxicity – D-galactosamine – Resveratrol

This work was supported by grants IGA MZ ČR NR/9379-3/2007, VZ MSM ČR 0021620807, GA ČR 305/07/0061.

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Introduction and Goals: Hepatoprotection remains one of the major challenges of pharmacotherapy. A number of naturally occurring phytochemicals were reported to be cell protectors. Among them, resveratrol (trans-3,5,4'-trihydroxystilbene) has been identified as a polyphenolic compound

with antioxidative, anti-inflammatory, phytoestrogenic, cardioprotective, anti-cancer activities. The aim of this project was to analyze the resveratrol's potential hepatoprotective effects on D-galactosamine (D-GalN)-induced hepatotoxic injury *in vivo* and *in vitro* in hepatocyte culture and bioreactor.

Methods After 30 min pretreatment with or without resveratrol (RES, 1–15 μM), rat hepatocytes were cultivated with D-GalN (5 mM) for 24–48 hr. In another cellular system, freshly isolated hepatocytes were immobilized in agarose thread bioreactor and perifused with appropriate drugs for 5 hr. Hepatocyte viability and mitochondrial activity were evaluated by ALT leakage, urea synthesis and MTT test. Apoptosis was estimated by caspase-3-like activity, cytosolic cytochrome c content and morphologically using Annexin-V/propidium iodide staining. NO production was determined as medium nitrite levels. Rat liver injury was induced by i.p. application of D-GalN (800 μg/kg) + lipopolysaccharide (LPS, 0.5 μg/kg) that followed RES (10 μg/kg) pretreatment. Liver damage was determined by biochemical and the histopathological examination.

Results RES reduced D-GalN-induced hepatotoxic effects in short term experiments (5 hr). D-GalN reduced urea biosynthesis of perifused hepatocytes that was increased by RES. Morphologically; there were ameliorations in apoptotic and necrotic markers under RES pretreatment both in hepatocyte bioreactor and *in vivo*. However, these hepatoprotective effects of RES were not observed in hepatocyte culture. On the contrary, RES dose-dependently aggravated D-GalN-induced apoptotic cell death of cultured hepatocytes as evidenced by significantly enhanced caspase-3-like activity, cytochrome c release, mitochondrial dehydrogenase activity and number of Annexin-V positive cells.

Conclusion Depending on the hepatotoxic model used, RES exerts cytoprotective properties in the liver *in vivo* and in short term cellular system on the one hand and proapoptotic activities in hepatocyte culture on the other hand. Moreover, it seems that the effects of RES on D-GalN-induced hepatotoxicity act through other mechanism(s) beside modulation of NO production. Since RES has antioxidant features, a possible explanation concerning these controversial results is related to the local reactive oxygen species generation that is variable under different experimental conditions. It can be supported by our findings of hepatoprotective activities of RES against oxidative stress induced by tert-butylhydroperoxide in perifused hepatocytes. In summary, RES effects obtained *in vitro* cannot be simply extrapolated to *in vivo* situation but they should be re-evaluated under various experimental conditions with emphasis on different molecular events.

Expression of Peroxisome Proliferator-activated Receptors Is Differentially Modulated in Daunorubicin-Induced Cardiomyopathy: the Effect of Treatment with ACE Inhibitor

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Key words: Peroxisome proliferator-activated receptors – Daunorubicin cardiomyopathy – ACE inhibitors

This study was supported by the grant of Slovak Society of Cardiolology 2007.

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Introduction Peroxisome proliferator-activated receptors (PPARs) are transcription factors crucially involved in the regulation of cardiac lipid metabolism and their abnormal expression might lead to cardiac dysfunction. Anthracycline-induced cardiomyopathy is characterized by impaired cardiac energetics, but the state of PPAR system has not been studied yet. Treatment with ACE inhibitors has been shown to retard progression of anthracycline cardiomyopathy, however mechanisms are unknown. In the present study we examined cardiac expression of three PPAR receptors isoforms: PPARα, PPARβ/δ and PPARγ in daunorubicin-induced cardiomyopathy and the effect of ACE inhibitor enalaprilate.

Methods Wistar rats were administered either vehicle or daunorubicin (DAU, 3 mg/kg, i.p., every 48 h), or daunorubicin and enalaprilat (DAU+ENA, 5 mg/kg, i.p., every 12 h) for two weeks. Isoprenaline-induced cell shortening was measured in isolated cardiomyocytes by confocal microscope. Left ventricles were snap frozen and processed for RNA isolation and mRNA expression measurements by real-time PCR using β-2-microglobulin as a standard.

Results Enalaprilate prevented daunorubicin-induced cell shortening dysfunction. The expression of PPARα was increased in DAU rats by 143 $\pm$ 20% when compared to controls, whereas the expressions of PPARβ/δ and PPARγ were unchanged. PPARα expression remained elevated in DAU+ENA by 162 $\pm$ 18% of control levels. Enalaprilat also increased the expression of PPARβ/δ by 175 $\pm$ 20% as compared with controls, while showing no effect on PPARγ mRNA level (Figure 1).

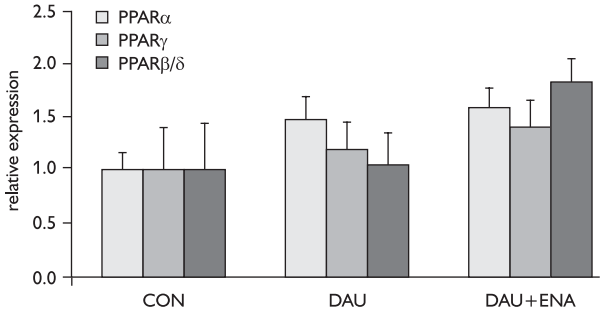

Figure 1 – Expression of cardiac PPAR receptors in three experimental groups. Enalaprilat upregulates PPARβ/δ and PPARα.

Conclusion In daunorubicin cardiomyopathy, the expression of PPARα receptor is increased, which may contribute to cardiac metabolic and functional dysfunction. ACE inhibitor enalaprilat failed to improve functional dysfunction and PPARα receptor upregulation, whereas it specifically increased PPARβ/δ expression. Therefore, modulation of PPARα receptor expression is associated with daunorubicin-induced cardiac dysfunction and may provide novel therapeutic target in anthracycline cardiomyopathy.

Pupillometric Biomarkers of Tramadol Effect in Healthy Subjects

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Key words : Infrared pupillography – Miotic effect – Tramadol – Pharmacodynamics

This study was supported by grants MSM ČR No. 0021620820 and 0021620849.

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Introduction Opioid and non-opioid actions of tramadol interact with the physiological mechanisms that regulate pupillary diameter in man. The main objective of the presented study was to test the different parameters of pupillography as a potential biomarker of tramadol efficacy.

Methods Healthy male and female subjects (n=66, 38 females and 28 males) participated in the study after they had given written informed consent. Mean age (± SD) of the subjects was 23.2 ± 4.9 years and body mass index

22.1 ± 2.7 kg/m^2. The volunteers were given tramdol drops orally in the dose of 0.7 mg/kg. The study drug was administered with 150 ml of water. Pupillography was performed using monocular infrared pupillograph "Pupilscan II$^{(TM)}$" in a quiet and fully darkened room. Pre-dose and 2 hour post-dose measurements were done on the right eye of the volunteers after at least 5 minutes of adaptation to the light conditions.

Results Pupillographic target parameters are depicted on the Figure 1.
We have observed significant effect of tramadol on all the static (initial baseline pupil diameter) and dynamic pupillometric parameters with the exception of reflex amplitude (RA) (Table 1). The difference of initial diameter pre-, and post-dose showed the highest sensitivity to detect the drug effects.

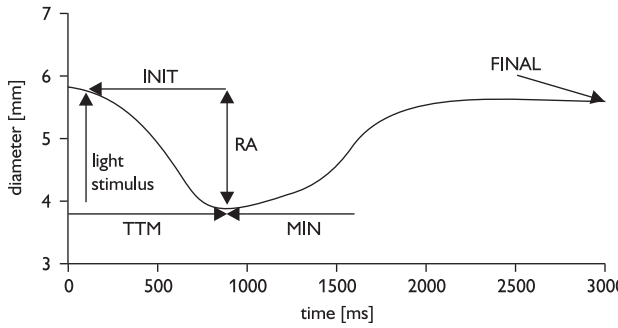

Figure 1 – Target pupillographic parameters. INIT – initial; MIN – minimum; FINAL– final diameter; TTM – time to minimum; RA – reflex amplitude.

Table 1 – Mean (SD) values of target parameters.

Parameters	Mean (SD) pre-dose	Mean (SD) post-dose	Difference (SD)	p (Sign – rank – test)
INIT (mm)	7.78 (1.34)	7.29 (1.14)	0.5 (1.14)	0.005
MIN (mm)	5.85 (1.38)	5.50 (1.22)	0.34 (1.34)	0.040
FINAL (mm)	7.03 (1.27)	6.96 (1.12)	0.39 (1.26)	0.040
RA (mm)	1.78 (0.56)	1.82 (0.60)	-0.02 (0.63)	NS
TTM (ms)	0.80 (0.09)	0.77 (0.11)	0.03 (0.09)	0.006

INIT – initial diameter, MIN – minimal diameter, FINAL – final diameter (after stimulus), RA – reflex amplitude, TTM – time to minimal diameter

Conclusions Pupil reaction measured by infrared pupillometry was useful non-invasive biomarker for pharmacodynamic effects of tramadol. The initial pupillary diameter was the most sensitive pupillographic parameter.

Acknowledgement The authors would like to thank Michael Friedrich Böttcher for Pupillscan II.

 Prague Medical Report / Vol. 109 (2008) Suppl., p. 78–79

The Affinity of Receptor Specific Peptides ^{177}Lu-DOTA-NOC and ^{177}Lu-DOTA-TATE to Somatostatin Receptors *in vitro*

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Key words: Somatostatin receptors – Internalization – Externalization – Somatostatin analogs

The study was supported by the grant No. 305/07/0535 of the GA ČR.

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Introduction The uptake of radiolabelled somatostatin analogs by tumour cells through receptor-mediated internalization is a critical process for the in vivo targeting of tumoural somatostatin receptors. Five human somatostatin receptor subtypes (sst_1 – sst_5) are known to be overexpressed to some degree on various tumours, sst_2 being the most important one. Clinically used somatostatin based radiopeptides target exclusively sst_2. Earlier studies have shown that modification of the octapeptide octreotide in positions 3 and 8 may result in compounds with increased somatostatin receptor affinity that display improved uptake in somatostatin receptor-positive tumours. In this study we analyzed an internalization of ^{177}Lu-DOTA-Tyr^3-octreotate (^{177}Lu-DOTA-TATE) and ^{177}Lu-DOTA-1-Na1^3-octreotide (^{177}Lu-DOTA-NOC) to rat pancreatic tumour cells *in vitro*. Whereas radiolabelled DOTA-TATE with the affinity only to sst_2 is clinically used at present, radiolabelled DOTA-NOC represents a new receptor-specific peptide with an extended spectrum of affinity also to sst_3 and sst_5.

Methods AR42J (from ECACC) cells were grown in RPMI-1640 (suplemented with 2 mM glutamine and 10% fetal calf serum) in air containing 5% CO_2 at 37 °C. Subculturing was performed employing a trypsin/EDTA solution. On the day of the experiment, the cells were treated with trypsin/EDTA solution and concentrated to 1.10^6 cells per 1 ml of internalization medium (RPMI-1640 supplemented with 2 mM glutamine and 1% fetal calf serum) per epppendorf tube. Incubation was started by addition 1ng of radiolabelled peptide per tube. Cells were incubated at 37 °C in triplicates for the indicated time periods. Cellular uptake was stopped by removal of the medium and washing of the cells with ice-cold PBS two times.

Thereafter the cells were incubated twice at ambient temperature in acid wash buffer (50 mM glycine buffer pH 2.8, 0.1 M NaCl) for 5 min. Cells were lysed by treatment in 1M NaOH and cell radioactivity collected (internalised radioligand fraction).

Results and Conclusions There is a little difference between the internalization range of peptides as seen on Figure 1. Values are expressed as specific internalization (percentage of dose added to 1 million cells at 1.5 nM concentration) and are result of three independent experiments with triplicates in each experiment. The AR42J rat pancreatic tumour cell line is known to express sst_2 receptors. It may be the reason why the both radiopeptides under study are internalized to these cells almost in the same range.

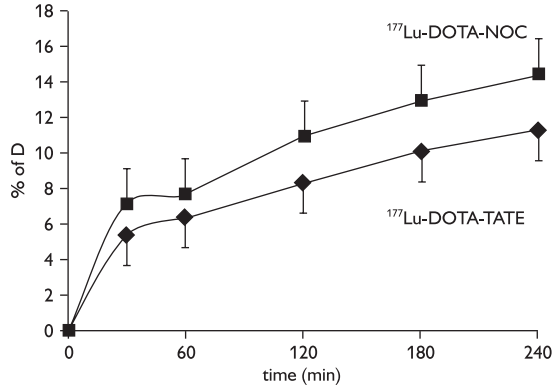

Figure 1 – Comparison of internalization rate of peptides into AR42J cells. Values are expressed as specific internalization (percentage of added dose).

The Impact of Pharmacogenetics in Therapy of Malignat Diseases

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Key words: Polymorphisms – MDR1 – Leukemia

This research project was supported by the grant number MZ SR 2005/46-VOUKE-01 from Ministry of Health of the Slovak Republic and by grant VEGA 1/3372/06.

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Introduction The impact of genetic background in childhood acute lymphoblastic leukemia (ALL) has not yet been fully understood. P-glycoprotein (Pgp), the gene product of MDR1, confers multidrug resistance to a number of antineoplastic agents. The expression of multidrug resistance gene MDR1 and its gene product P-glycoprotein (Pgp), is being widely studied in leukemias and solid tumors. Pgp is a transmembrane protein that acts as an efflux pump in an adenosine triphosphate (ATP)-dependent fashion. Pgp recognizes and transports a variety of drugs including chemotherapeutic agents, antibacterials, immunosuppressants, calcium channel antagonists or HIV protease inhibitors. In general, Pgp substrates are hydrophobic, organic cations at physiological pH, contain one or more aromatic rings and have molecular weight > 400 g.moL^{-1} A silent mutation in the exon 26 (C3435T) has been associated with altered expression and function of Pgp in tissues. Pharmacogenetic pretherapeutic screening of single nucleotide polymorphisms (SNP) in relevant genes, which encode for proteins that interact with anticancer drugs, may lead to identification of specific populations predisposed to poor drug responses and drug toxicity. Pharmacogenetics for individualized cancer chemotherapy is, therefore, an important area of investigation.

Methods Thirty-four children with newly diagnosed ALL were included in the present study. To detect whether C3435T MDR1 polymorhism is associated with ALL outcome, DNA isolated from peripheral blood or bone marrow was studied by polymerase chain reaction-restriction fragment-length polymorhism (PCR-RFLP) assay. The statistical analysis (Kaplan-Meier and Wilcoxon test) of factors influencing clinical outcome in our ALL patients revealed that CC genotype carriers had significantly shorter event-free survival (EFS) time (0.20 vs. 0.85; $P=0.0004$). At the same time, they had significantly (Mann-Whitney U test) higher IC_{50} for methylprednisolone as assessed by *in vitro* MTT test compared to patients with CT and TT genotype ($P=0.035$).

Results and Conclusions To conclude, preliminary results of our study provide evidence that C3435T MDR1 polymorphism could affect the clinical outcome of childhood ALL. The carriers of CC genotype are supposed to have worse prognosis and response to treatment in comparison with CT/TT carriers. To confirm these results, increase in the number of patients in individual groups will be necessary.

Correlation of Polymorphism C3435T of the MDR-1 Gene and Progression-free Survival Time in Women with Breast Cancer

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Key words: Breast cancer – MDR1 – Gene polymorphism

Supported by the research project MZ SR 2005/46-VOUKE-01 and grants VEGA 1/2282/05 and 1/3372/06.

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Introduction The human multidrug-resistant gene (MDR1) encodes P-glycoprotein (Pgp). It is a transmembrane protein which acts as an efflux pump increasing efflux of variety of natural cytotoxic agents from the cell. This effect confers resistance to a number of anticancer drugs of natural origin in malignant tumor cells. Single nucleotide polymorphisms (SNPs) in MDR1 gene are usually associated with phenotypic variation in Pgp expression levels of different tissues. In normal tissues SNPs may alter the physiological protective role of Pgp. On the other hand in pathological tissues as malignant tumors they may result in tumor cell resistance and chemotherapy failure.

Breast cancer is the most common cancer in women in Europe. Systemic breast cancer treatment includes cytotoxic, hormonal and immunotherapeutic substances. Even the systemic treatment is active at the beginning of therapy in large majority of primary breast cancers, progression occurs after a variable period of time. The purpose of the following study was to examine the MDR1 C3435T polymorphism in women with breast cancer systemicaly treated with anthracyclines or taxanes and/or tamoxifen-based adjuvant hormonal therapy and evaluate the association between the polymorphism and progression free survival.

Methods Sixty one breast cancer women were studied for presence of the MDR1 C3435T polymorphism. DNA was extracted from peripheral

blood lymphocytes using standard extraction method. Polymerase chain reaction-restriction fragment length polymorphism was used for detection of C3435T SNP.

Results and Conclusions We obtained CC, CT and TT genotype frequencies in breast cancer patients as 20.3%, 47.8% and 31.9%, respectively. In the control group, frequencies of genotypes were found as 31.0% for CC, 47.8% for CT and 21.2% for TT. The progression-free median survival time of women in a subgroup analysis based on MDR1 genotypes with CC genotype was 15 months and with CT, TT genotypes 34 months. This differences between C or T alleles were found to be statistically significant (long rank, Mantel-Cox test, $p=0.0332$).

To indicate an individual treatment with increased efficacy and low toxicity, selecting therapies based on the patient and the clinical and molecular characteristics of the tumor is necessary. Despite advances in early detection and understanding of the molecular bases of breast cancer biology, approximately 30% of all women breast cancer have reccurent disease. In adjuvant therapy anthracycline-based regimens are preferred with the addition of noncross-resistant agents as taxanes in some women to increase the survival rate. In this therapeutic mode, SNPs in MDR1 gene may affect progression-free survival rate, as both anthracyclines and taxanes are substrates of Pgp. Some studies did not find significant statistical difference between clinicopathological parameters and MDR1 phenotype. However, our results demonstrated significant difference in progression-free median survival time in women with CT, TT genotypes comparing to CC genotype. These results are in agreement with previously published findings of MDR1 polymorphism in preoperative chemotherapy with anthracyclines or these agents combined with taxanes. The authors found a significant correlation ($p=0.029$) between clinical complete response to preoperative chemotherapy and the T/T genotype. In conclusion, the results of the present study demonstrated an impact of MDR1 genotype on progression-free survival in women with breast cancer.

Proapoptotic and Antiangiogenic Effects of Selected Chalcones

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Key words: Chalcones – Apoptosis – Angiogenesis inhibition

This work was supported by the Slovak Research and Development Agency under the contract No. APVV-0325-07 and by VEGA grants 1/4236/07 and 1/3365/06.

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Introduction Chalcones are precursors of flavonoids in their biosynthetic pathway. Variety of biological activities have been demonstrated for these compunds such as antiinflammatory, analgesic, antiviral, antibacterial, gastroprotective, antioxidant as well as cytotoxic properties. Moreover, there is also some evidence about their antiangiogenic effects. In the present study, we have investigated whether newly synthetized chalcones possess an antitumour and/or antiangiogenic activity.

Methods *chalcones* (4-hydroxychalcone (1), *E*-2-(X-benzylidene)-1-tetralones (2a, 2b) and *E*-2-(4'-methoxybenzylidene)-1-benzosuberone (3) were synthetised by Perjesi and co-workers. *Jurkat cells* were maintained in RPMI 1640 medium with Glutamax-I supplemented with 10% foetal calf serum in the atmosphere 5% CO_2 in humidified air at 37°C. *Cytotoxic effect* of the tested compounds wase studied by using colorimetric microculture assay with the MTT end-point. *Cell cycle distribution* in cells treated with the tested agent was analyzed by propidium iodide DNA staining using Cycle TEST^{TM} PLUS DNA Reagent Kit (Becton Dickinson, USA). *Annexin V/PI staining* was performed according manufacturer's instructions. *DNA fragmentation* was assayed by agarose gel electrophoresis. Antiangiogenic effect of compound tested was sudied using human umbilical vein endothelial cells (HUVEC). *Endothelial cell migration assay* – the migratory activity of HUVECs was assessed using a wound healing assay. *In vitro* angiogenesis assay – the effect of the studied compounds on capillary tube formation by HUVECs was performed using the Fibrin Gel *In Vitro* Angiogenesis Assay Kit according manufacturer's instructions. *Gelatinase zymography* – Matrix metalloproteinases (MMP) released into conditioned media were determined by gelatinase zymography. *VEGF Quantification* – VEGF protein released into the conditioned medium was measured by using a commercial ELISA kit.

Results and Conclusions From chalcones tested only compound 3 possess significant cytotoxic effect on cancer cells. Incubation of Jurkat and HeLa cells with compound 3 at 1 mmol/L for 72h caused 87 and 45% reduction in cell survival, resp. Furthermore, it caused initial G_2/M arrest in both cell lines followed by an

increase in the proapoptotic sub-G0/G1 fraction. Apoptosis was also confirmed by both methods.

The cytotoxic effect of compound 3 against HUVECs was concentration-dependent and cell survival significantly decreased at $c=10^{-4}$–10^{-6} mol/L. It also completely inhibited CTF by HUVECs in non-toxic concentrations (10^{-7}–10^{-8} mol/L). Moreover, this chalcone in the same concentrations effectively block also ECM. In biochemical analysis, chalcone 3 treatment of HUVEC for 24 h resulted in a concentration-dependent decrease in the secretion of matrix metalloproteinase (MMP-9). Inhibitory effect on MMP-2 activity was observed only at the highest concentration. Furthermore, exposure of HeLa cells (cervix cancer) to chalcone 3 resulted in a dose-dependent decrease in the secreted VEGF level in conditioned media.

Other chalcone tested possess antiproliferative/antiangiogenic effects only in the highest concentration used (10^{-4} mol/L).

The present study demonstrates antiproliferative/antiangiogenic properties of chalcone 3. Further studies are necessary to elucidate its mechanism of action, nevertheless, this compound might have a potential to enter pre-clinical trials as a new anticancer drug.

Cardiovascular Effects of Dexamethasone in Experimental Model of Meconium Aspiration Syndrome (MAS)

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Key words: Meconium aspiration – Glucocorticoids – Cardiovascular effects

This project was supported by the grant projects VEGA No. 1/2306/05 and 1/0061/08 and project of ESF No. SOP LZ 2005/NP1-027.

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Introduction Aspirated meconium may in newborns lead to airway obstruction, surfactant inactivation, inflammation, and pulmonary vasoconstriction. Glucocorticoids may improve lung function, but little is known about their side effects. Aim of this study was to evaluate immediate and early effects of

dexamethasone on blood pressure (BP), heart rate (HR), and heart rate variability (HRV) in animals with MAS.

Methods Adult rabbits with intratracheal (i.t.) instillation of human meconium or saline (4 ml/kg) intravenously (i.v.) received two doses of dexamethasone (each of 0.5 mg/kg) or corresponding volume of saline 0.5 and 2.5 h after i.t. meconium or saline instillation. Animals of all groups (Mec+Dex, Sal+Dex, Mec+Sal, and Sal+Sal) were oxygen-ventilated for 5 h after the first dose of treatment. BP, HR, and short-term HRV were analyzed during and immediately after i.v. dexamethasone or saline administration, and within 5 h after the first dose of treatment.

Results and Conclusions In meconium-instilled animals, dexamethasone significantly decreased HR and increased BP and HRV parameters, and caused cardiac arrhythmia during and immediately after the administration. In saline-instilled animals, dexamethasone had no immediate effects on evaluated parameters. Although acute influence of dexamethasone almost disappeared within 30 min., HR was lower and HRV parameters, particularly total spectral power, spectral power in high-frequency band, and mean squared successive difference (MSSD) were higher till the end of experiment in both dexamethasone-treated groups ($P<0.05$, Figure 1). If systemic glucocorticoids are used in the treatment of MAS, cardiovascular side effects should be considered.

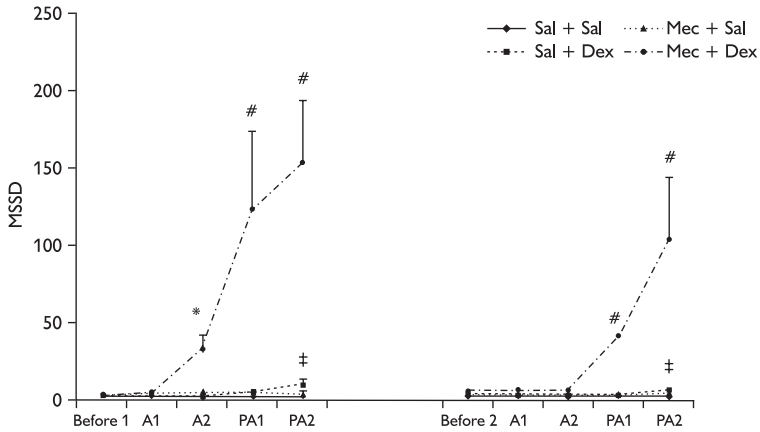

Figure 1 – MSSD (expressed in ms^2) before the first and second (Before 1, 2) dose of treatment (dexamethasone or saline i.v.) and during two intervals of 2.5 min. of treatment administration (A1 and A2) and during two intervals of 2.5 min. after finishing the administration (PA1 and PA2). For Mec+Dex vs. other groups: $^{*}P<0.05$, $^{\#}P<0.01$; for Sal+Dex vs. Sal+Sal and Mec+Sal, $^{‡}P<0.05$. Data are expressed as means±SEM.

 Prague Medical Report / Vol. 109 (2008) Suppl., p. 86–88

Influence of Selective PDE Inhibitors on Cough and Airway Reactivity in Healthy and Ovalbumin-sensitized Guinea Pigs

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Key words: Phosphodiesterase – Cough – Citalopram – Cilostazol – Zaprinast – Vinpocetin – Airway hyperresponsiveness

This project was supported by grants VEGA No. 1/0072/08, No. 1/3375/06, and Grant of MZ SR No. 2005/13-MFN-05.

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Introduction There are several groups of drugs used in the therapy of cough. However, chronic cough remains still a hot clinical problem, as the adverse effects of most effective antitussives limit their use and stimulate further studies of mechanisms and possible pharmacological modulation of cough in various clinical conditions and diseases. One of such ways could be a suppression of inflammation in patients with airway diseases associated with cough and airway hyperresponsiveness. Thus, the inhibition of phosphodiesterase (PDE) could be a benefit. The aim of this study was to evaluate the influence of selective inhibitors of PDE on cough and airway reactivity in healthy and ovalbumin-sensitized guinea pigs.

Methods Cough and airway reactivity was evaluated in non-anaesthetized guinea pigs in double chamber whole body plethysmograph. The cough was evoked by inhalation of citric acid aerosol (AC; 0.6 mol/l) and trained observer visually counted and recorded number of coughs during AC nebulization (2 min) as well as after finishing the nebulization (2 min). As a marker of *in vivo* airway reactivity specific airway resistance was measured after 2 min lasting nebulization of AC and histamine aerosol (10^{-6} mol/l). For *in vitro* airway reactivity organ chambers were used. Tissue strips from trachea and lungs were exposed to cumulative doses of histamine or acetylcholine (10^{-8}–10^{-3} mol/l) and contractile responses were recorded. In blood, count and differential count of white blood cells (WBC) were evaluated. All parameters were measured in healthy as well as ovalbumin-sensitized guinea pigs before and after intraperitoneal administration

of vinpocetin (selective PDE1 inhibitor), cilostazol (selective PDE3 inhibitor), citalopram (selective PDE4 inhibitor) or zaprinast (selective PDE5 inhibitor) at a dose of 1 mg/kg.

Results and Conclusions Ovalbumin-sensitization of guinea pigs caused significant increase in number of cough efforts, specific airway resistance, as well as significantly increased tracheal and lung tissue reactivity to histamine and acetylcholine. Pre-treatment with selective inhibitor of PDE1 (vinpocetin), PDE3 (cilostazol) and PDE5 (zaprinast) decreased the number of cough efforts in healthy guinea pigs, whereas vinpocetin, citalopram (PDE4 inhibitor) and zaprinast significantly suppressed cough in ovalbumin-sensitized animals. Significant decrease of *in vivo* airway reactivity in sensitized animals was observed only after pre-treatment with cilostazol and zaprinast, suggesting that PDE3 and PDE5 participate in inflammatory processes and airway hyperresponsiveness evoked by repeated exposure to ovalbumin (Figure 1). Contrary, *in vitro* testing confirmed significant suppressing effect on airway reactivity of cilostazol and citalopram in both healthy and ovalbumin-sensitized guinea pigs. Sensitization with ovalbumin led to significant increase of WBC count in blood, with predominant increase in relative count of neutrophils, monocytes and eosinophils. Both cilostazol and citalopram decreased the count of monocytes and neutrophils, confirming their antiinflammatory potential. Inhibition of PDE could be a valuable target for

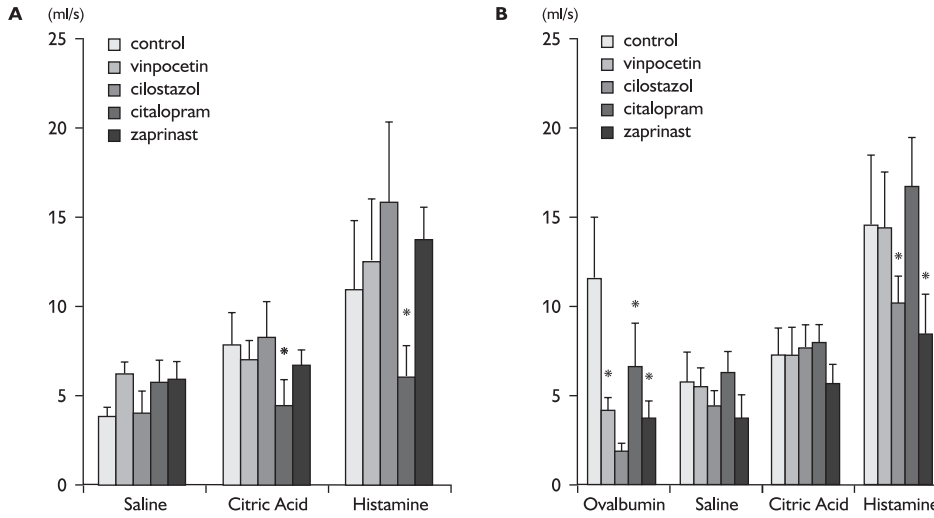

*Figure 1 – Specific airway resistance after inhalation of saline, citric acid and histamine in healthy (non-sensitized) guinea pigs (A) and ovalbumin-sensitized guinea pigs (B) before (control) and after administration of selective PDE inhibitors. (*p<0.05 vs. control).*

influencing cough and airway hyperresponsiveness in diseases associated with inflammation. Selective inhibitors of PDE (especially inhibitors of PDE3 and PDE4) could be preferred to non-selective (e.g. methylxanthines), as they are able to influence the pathomechanisms of inflammatory airway disease under lower incidence of severe adverse effects. However, their therapeutical potential as antitussive and antiinflammatory drugs needs to be confirmed in further experimental and clinical studies.

Pharmacologic Regulation of Heme Oxygenase 1

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Key words: Heme – Bilirubin – Carbon monoxide – Antioxidant

This work was supported by grant IGA MZ ČR Nr. 9366-3.

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Introduction Heme oxygenase (HO), the rate limiting enzyme in the heme catabolic pathway, catalyzes the oxidative degradation of heme to form equimolar amount of iron, carbon monoxide (CO) and biliverdin, which is rapidly reduced to bilirubin by biliverdin reductase. All these products are important bioactive molecules.

Bilirubin has been considered as a toxic waste product because neonatal hyperbilirubinaemia could cause bilirubin encephalopathy. However, recent findings show bilirubin to exert strong antioxidant effects at physiological and/or mildly elevated serum concentrations. Similarly in CO, the attention shifted from a toxic gas produced during the incomplete combustion of organic materials to an essential endogenous messenger displaying antiapoptotic, antiinflammatory and cytoprotective actions. Transient elevations of intracellular iron can trigger the synthesis of ferritin leading to the exertion of strong cytoprotective actions, which may also affect cellular redox potential and gene expression processes within the cell.

Results There are two structurally related HO izoenzymes, the inducible HO-1, also known as the heat shock protein 32 (HSP32), and the constitutive HO-2.

Expression of HO-1 is inducible or repressible, depending on cell or tissue types, while expression levels of HO-2 are maintained within narrow ranges.

Moreover, the human HO-1 gene promoter contains (GT)n repeat polymorphism and single nucleotide polymorphisms modulating the level of HO-1 expression to stress. The presence of longer (GT)n repeats exhibits lower transcriptional activity and is associated with susceptibility to various pathologic conditions, e.g. coronary artery disease, lung emphysema or certain types of cancer.

Since HO-1 is upregulated as a protective mechanism in response to various stress stimuli (hydrogen peroxide, cytokines, heme, endotoxin, heavy metals, UV radiation etc.), targeted induction of this stress- response enzyme may be considered as an important therapeutic strategy for the protection against inflammatory processes and oxidative stress.

Statins were shown to induce heme oxygenase *in vitro* and *in vivo* and a tissue specific HO induction in the heart was suggested to play a crucial role in antioxidant and cardiprotective effects of these drugs. Upregulation of HO-1 plays a key role in the antiatherogenic effect of another cholesterol- reducing drug, probucol. A very interesting and still incompletely understood aspect of HO-1 regulation is its inducibility by nitric oxide (NO) and NO releasing agents. Interestingly, aspirin appears to exert part of its antiinflammatory effect *via* NO-mediated induction of HO-1. Although HO-1 is up-regulated in response to oxidative stress, certain polyphenols with antioxidant activity or thiol modyfying agents can display a similar effect. This group includes a broad range of different chemicals, such as curcumin, caffeic acid phenethyl ester, rapamycin, carnosol or resveratrol. Also pharmacologic doses of insulin have been reported to induce HO-1 in renal cells. This induction may explain a renoprotective effect of insulin in addition to its effect on circulating glucose concentration.

HO-1 is upregulated not only by its substrate, heme, but also by its reaction product CO. This effect together with a biologic importance of CO led to synthesis of CORMs (CO releasing molecules), transition metal carbonyls with ability to liberate CO in a biological environment.

Some metaloporphyrins inhibit HO activity and are potential compounds for the treatment of neonatal jaundice. HO-1 might be also repressed in cultured human cells under the thermal stress, hypoxia, by the treatment with interferon γ or desferoxamin. In rodent cells, situation might be completely different and HO-1 might be induced. We have recently showed that HO activity is decreased in primary rat hepatocytes treated with bile acids.

The pharmacologic targeting and regulation of HO-1 has been intensively studied and a lot of aspects need to be clarified. It is important to distinguish a beneficial induction of HO-1 from a response to stress caused by toxic concentrations of particular drug. It is also necessary to link upregulation of HO protein with HO activity and concentration of bioactive molecules, as the induction of enzyme does not automatically mean the increase in the products.

Conclusion Taken together, heme oxygenase and particularly its metabolic products emerged to play a pivotal role in cellular defence and maintainence of cellular redox homeostasis. As induction of HO-1 expression appears to be of a therapeutic importance, novel compounds targeting HO-1 are identified and provide us a hope for future treatment of a number of diseases and pathological conditions.

Monitoring of Potential Cardioprotective Effect of Midazolam (Pilot Study)

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Key words: Heart ischaemia-reperfusion – Midazolam

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Introduction Midazolam is a benzodiazepine derivative. It has powerful anxiolytic, amnestic, hypnotic, anticonvulsant, skeletal muscle relaxant and sedative properties. It is considered a fast-acting benzodiazepine, with a short elimination half-life. It is therefore a very useful drug to use for short minor procedures such as dental extraction. Midazolam was first synthesized in 1976 by Fryer and Walser.

Aim of the study The aim of the study was to monitor the effects of midazolam during heart perfusion of laboratory rat.

Material and Methods This study was performed on 20 male Wistar SPF laboratory rats of the same age and comparable weight. After a recovery period, the animals were divided randomly into 2 groups (n = 10). The first group – treated group – received midazolam in a single dose 0.5 mg/kg by i.p. injection. The second group – the placebo group – received only normal saline solution also by i.p. injection. The rats were anesthetized with an i.p. injection of anaesthetical mixture. After the i.p. heparine injection of 500 IU dose, the hearts were excised and perfused. In all experiments, the modified Langendorff method and the universal apparatus Hugo Sachs Electronic UP 100 (Germany HSE) were used.

Schedule: stabilization/ischaemia/reperfusion proceeded at intervals of 20/30/60 min. Biomechanical parameters from isolated heart: left ventricle pressure (LVP), end-diastolic pressure (LVEDP), contractility (dP/dt_{max}) were measured using a ball filled with liquid (8–12 mmHg), inserted through the left atrium in the left ventricle connected to the analog convertor.

Results In hearts from placebo animals, LVP recovered up to 61 ± 7% of pre-ischemic values at the end of the reperfusion. In the midazolam pretreated animals, the hearts showed significantly better post-ischaemic recovery, reaching LVP values of 92 ± 6% at the end of the reperfusion. In hearts from placebo animals LVEDP rose from 10.0 ± 0.5 to 43 ± 4 mmHg after 60 min of reperfusion. This increase was diminished in the hearts from the midazolam pretreated animals at the end of reperfusion. The pretreatment with midazolam improved $+dP/dt_{max}$ recovery during reperfusion to 91 ± 8% after 60 min of reperfusion. These values were significantly greater than those obtained from placebo hearts.

Conclusion From the results of our experiment it can be deduced that the administration of midazolam in the laboratory rats has cardioprotective potential against ischaemia-reperfusion induced injury in rat hearts. This effect is demonstrated in the functional parameters of hearts, LVP, LVEDP and $+dP/dt_{max}$.

From recent studies there is increasing evidence that cardioprotection by anaesthetic agents can be elicited in the clinical setting and may add to other organ protection strategies. Thus, it is conceivable that the choice of anaesthetic drug may have an impact on patient outcome in ischaemia-reperfusion situations. However, this still has to be confirmed by large studies that examine definitive outcome parameters.

Chitosan Derivates: *in vitro* Determination of Hepatotoxicity

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Key words: Cytotoxicity – Hepatotoxicity – Chitosan derivates – Chitosan – Viability – *in vitro*

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Introduction Chitosan, (poly-D-glucosamine), is a natural polymer derived from chitin, the second most abundant polysaccharide after cellulose. According

to the growing number of recently published scientific articles, it can be deduced, that chitosan is a perspective material of the extensive potential for various applications. Although there exist many scientific studies being engaged in various modifications, a detail mechanism of activity on the molecular level has not yet been discovered.

The aim of this work was to determine the level of toxicity of six different chitosan derivates in vitro. The derivates were variously substituted by antituberculotic drugs due to reduce their hepatotoxicity.

Methods The CellTiter-Blue cell viability assay was used for hepatotoxicty determination in vitro. We have choose to use HepG2 cells for the experiment. The cells were seed into 96-well plate and they were cultivated 24 hours to sit down. Then the tested compounds were added dissolved in cell culture medium. Incubation time with tested compounds was 48 hours to catch even low rates of toxicity (decrease od viability). The CellTiter-Blue reagent was added after the incubation. Cells were let to metabolise the reagent for 2 hours, then the plate was analyzed with fluorimetric reader (560 nm excitation and 590 nm emision wave lenght). Gained data were converted to percent of viability according to control number of natural viable cells.

Results and Conclusions The experiment has shown that tested derivates of chitosan has low or no toxic effect on the hepatic cells. For details see Figure 1.

Acknowledgement Specials thanks to Tomáš Šimůnek, PharmD., PhD. and Pavel Bárta, MSc.

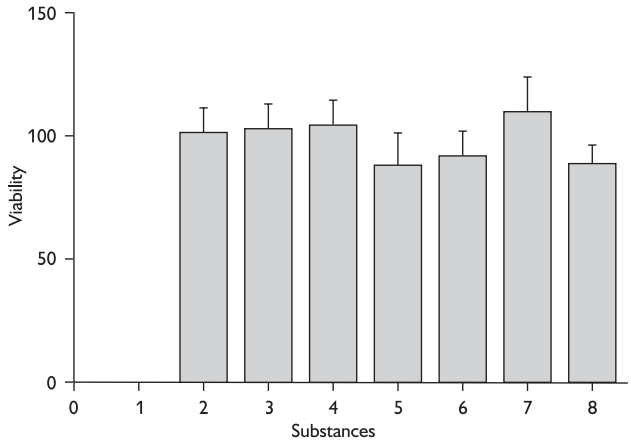

Figure 1 – Percentual viability of HepG2 cells treated with tested compounds.
1: Isoniasid 200 mM/l,
2: Cells with medium,
3: Chitosan1, 4: Chitosan2,
5: Chitosan3, 6: Chitosan4,
7: Chitosan5, 8: Chitosan6.

Altered Expression of Novel Components of Renin-angiotensin System in the Development of Spontaneous Hypertension

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Key words: Renin-angiotensin system (RAS) – Hypertension – Losartan – Real-time PCR – Kidney

This project was supported by the grantof Comenius University number UK/339/2007.

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Introduction The recent evidence shows that G-protein-coupled receptor Mas can hetero-oligomerize with the AT_1 receptor and thereby prevent its activation by angiotensin II (Ang II). Furthermore, Ang-(1-7), produced by cleavage of Ang II by angiotensin converting enzyme-2- ACE2, may activate Mas receptor and thus induce benefitial vasodilatory and antiproliferative effects. We studied expression of these novel components of renin-angiotensin-system (RAS) in heart and kidney of spontaneously hypertensive rats (SHR) and their potential modulation by perinatal AT_1 receptor blockade with losartan.

Methods Blood pressure was measured by tail-cuff in Wistar Kyoto rats (WKY, n=5), SHR (n=5) and SHR treated with losartan (20 mg/kg/day, p.o. twice daily) perinatally up to 9 weeks of age (SHR+LOS 0–9, n=5, dams during gestation/lactation period and pups from weaning at 4). At the age of 9 weeks, ACE, ACE2, AT_1 receptor and Mas receptor mRNA levels in renal cortex and ACE and ACE2 mRNA levels in left ventricles were determined by real-time PCR.

Results and Conclusions Perinatal losartan treatment (105±3 mmHg) prevented the rise in blood pressure (109±2 vs 149±2 mmHg in WKY and SHRs, respectively). Renal expression of ACE2 was upregulated more than two-fold in SHR rats when compared to WKY, however it was not changed in the heart (Table 1). Treatment with losartan led to additional increase in renal, but not affecting cardiac ACE2 expression. ACE expression was significantly lowered by 52 only in the heart of SHR rats, but unaffected by losartan treatment (Table 1).

In the kidneys of WKY, the expression of both AT_1 and Mas receptor were detected, while they were either substantially lowered (AT_1 receptor) or completely absent (Mas receptor) in SHR rats (Table 1). None of the later mRNA levels was affected by losartan treatment. Our results suggest that local RAS is regulated differentially in the kidneys and hearts of SHRs. Downregulation of ACE, AT_1 receptors and upregulation of ACE2 in the kidneys of SHR rats may represent mechanisms compensating for the local RAS activation. On the other hand, lack of expression of Mas receptor may participate in the development of spontaneous hypertension. Perinatal treatment with losartan fails to prevent these changes.

Table 1 – Relative expressions of novel components of renin-angiotensin system in kidneys and hearts of SHR rats perinatally treated either with vehicle (SHR) or losartan (SHR+LOS)

Relative expression		Kidney	
vs. WKY	WKY	SHR	SHR+LOS
ACE	1.00 ± 0.24	0.58 ± 0.17	0.40 ± 0.19
ACE2	1.00 ± 0.20	2.22 ± 0.35*	4.14 ± 1.23*
AT_1 receptor	1.00 ± 0.14	0.19 ± 0.04*	0.25 ± 0.02*
Mas receptor	1.00 ± 0.14	undetected	undetected
Relative expression		Heart	
vs. WKY	WKY	SHR	SHR+LOS
ACE	1.06 ± 0.14	0.48 ± 0.09*	0.50 ± 0.14*
ACE2	1.05 ± 0.14	0.91 ± 0.09	1.23 ± 0.23
AT_1 receptor	n.a.	n.a.	n.a.
Mas receptor	n.a.	n.a.	n.a.

*$p<0.05$

Hyperlipidemia in Patients after Kidney Transplantation

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Key words: Hyperlipidemia – Chronic renal failure – Immunosuppressive therapy – Kidney Transplantation – Hypolipidemic therapy

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Introduction High lipide levels play the most important role at cardiovascular risk by acceleration of the atherosclerosis. It depends not only on primary hypercholesterolemia or hypertriglyceridemia but could be caused by secondary increasing of those. Expansion of secondary hyperlipoproteinemia and worsening of the existing disorder threatens the patients after the kidney transplantation. The aim of this work was monitoring the hypolipidemic therapy after kidney transplantation.

Methods The followed file was set up by 30 patients after kidney transplantation (21 men and 9 women, age range 24–70 years at the year of transplantation 2005). All recipients were undergone an imunosuppresive tharapy, double or triple combined drugs (Prednison and Cyclosporin A or Tacrolimus, Prednison, Mycofenolat and Cyclosporin A or Tacrolimus). By the type of hyperlipoproteinemia there were 14 patients treated just by fluvastatin, 4 patients just by fenofibrat, 6 patients by combined therapy and 6 pacients without hypolipidemic therapy. Each biochemical parameter (Total cholesterol, Triglycerides, HDL-cholesterol and LDL cholesterol) was assigned three times – initial, one and two years after. The starting file was than divided by intial diagnosis which lead to the transplantation into three groups (glomerulonefritis – 15, congenitive renal diseases – 8 and others – 7 patients) and also by used triple-combination therapy (P/MF/CyA – 16 and P/MF/Tac – 11 patients). In all groups we monitored levels of biochemical parameters ment above.

Results There was faster worsening of renal functions in men, but there hasn't been found diferences in serum lipid level between men and women.

There is a noticable decrease of cholesterol and trigliceride level in the group of patients after kidney transplantation because of glomerulonefritis.

In the point of view of immunosuppressive therapy there was found better impact on lipid metabolism in the group treated by combination P/MF/Tac but just at the beginning of the therapy.

The progress of secondary hyperlipoproteinemia and worsening of the existing disorder is indicated by decreasing of the HDL-cholesterol level and increasing of level of triglycerides.

Discussion The fluvastatin and/or fenofibrat were used in the file. At present, there are some new drugs and combination of them to decrease lipide levels. In future, it would be interesting to compare other statines or other combined therapy to reduce cardiovascular risk in patients after kidney transplantation.

Conclusions The lipid-metabolism disorders are more than 20 years forefront the prevention of the cardiovascular diseases. As for patients after kidney transplantation, it is not just about drug treatment of secondary

hyperlipoproteinemia, but also about prevention of the graft rejection, which could be caused by nefrosclerosis or by sclerosis of arteria renalis, too. Hypolipidemic therapy contributes to the cardiovascular risk reduction in association with other precautions – low lipid diet, regular and periodic physical training and healthy life style.

Metabolomics and Monkeys – Tools for Understanding Type 2 Diabetes Mellitus

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Key words: Metabolomics – Diabetes

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Introduction The field of metabolomics is rapidly evolving and is presented with the arduous task of identifying and determining the concentrations of all metabolites (i.e., the metabolome) in a biofluid (e.g., urine, serum, saliva, or sweat), tissue extract, or organism. Making matters more complicated is the lack of metabolome annotation, particularly for mass spectrometry-based approaches, thus turning final structural determination into an often complicated and time-consuming process. However, current efforts such as the Human Metabolome Project and the Lipid Metabolites and Pathway Strategy (LIPID MAPS) have begun to shed some much needed light on the metabolome, and suggest it to be a vast, untapped resource for biomarker discovery. Additionally, characterizing the metabolome will be an essential addition to other "omics" disciplines such as genomics, transcriptomics, and proteomics, and the synthesis of all the "omics" data will be useful for generation of a true systems biology perspective of disease.

Type 2 diabetes mellitus (T2DM) is increasing at an alarming rate worldwide and is a major economic burden. Consequently there is an urgent need for non-invasive biomarkers of T2DM that can suggest disease onset at its earliest manifestation. Within the last five years, metabolomics-based biomarker discovery efforts for T2DM have been demonstrated using a variety of animal models (e.g., *db*/*db* mouse, Zucker diabetic rat) and analytical platforms (NMR, HPLC-MS, UPLC-QToF, and GC-ToFMS). These studies have reported clear proof-of-concept for metabolomics as a tool to discriminate normal from T2DM samples, yet their applicability to the T2DM human population remains untested.

While human studies are costly and, in most cases, less ideal due to the difficulty or impossibility of obtaining tissue samples for gene expression and other "omics" analyses, the rhesus macaque (*Macaca mulatta*) that develops T2DM spontaneously and naturally under highly consistent and healthy dietary conditions may serve as an ideal model. In addition to being closely related to humans genetically, the T2DM rhesus macaque exhibits the same phenotype and complications as T2DM humans.

Additionally, when conducting metabolomics studies, diet and other lifestyle-related factors should not be underestimated in their contribution to the metabolome. Understanding T2DM should begin with a model system in which these factors can be strictly controlled. Even the gut microbiota has been shown to play a pivotal role in defining an animal's metabolome.

Conclusion These few examples indicate that the metabolome is context driven, and therefore environmental factors must be precisely controlled and understood (as with laboratory-maintained animal models of disease) in order to sort out pathology driven features to identify possible progressive changes. The combination of the unique, spontaneously, and naturally T2DM rhesus macaque with the latest in metabolomics technology will likely provide a new view of the pathogenesis and progression of T2DM.

Pharmacogenetics of Nuclear Receptors in CYP3A4 Gene

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Key words: Constitutive androstane receptor – CYP3A4 – Nuclear receptor – Transcriptional regulation – SNP

This project was supported by the grant number GA ČR 303/07/0128 and IGA MZ NR/9206-3 (P.P.).

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Introduction Cytochrome P-450 CYP3A4 gene in transcriptionally regulated by several nuclear receptors including xenobiotic-activated Pregnane X Receptor (PXR) and Constitutive Androstane Receptor (CAR).

The presentation will briefly summarize our current knowledge of single nucleotide polymorphisms (SNPs) of PXR and CAR receptors in transactivation of CYP3A4.

In addition, we will present our data on the effect of four naturally occurring variants containing SNPs in ligand-binding domain (LBD) of CAR in ligand-activated transactivation of CYP3A4 gene. At the same time, we will present our results on ligand-mediated transcriptional potential of CAR3 nuclear receptor, which is the alternatively spliced variant of CAR nuclear receptor with in-frame five-amino acid insertion in the LBD.

Methods To evaluate ligand-dependent transactivation of CYP3A4 gene by polymorphic CAR receptor and its CAR3 variant, gene reporter assay using several luciferase reporter constructs with various promoter sequences of CYP3A4 gene and expression plasmids of polymorphic and variant CAR receptors were used.

Results and Conclusions We describe distinct effect of SNPs in LBD domain of CAR receptor in drug-mediated transactivation of CYP3A4. In addition, we demonstrate different properties of variant CAR3 receptor in both basal and drug-induced transactivation of CYP3A4.

We can conclude that pharmacogenetics of transcriptional factors and nuclear receptors should be considered as an important factor that can contribute to interindividual variability in both basal and inducible gene regulation of phase I. and II. metabolizing enzymes.

Occurrence of Cardiovascular Diseases Depending on Presence of Genetic Predisposition and Using of Combined Oral Contraception

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Key words: Thrombophilia – FV Leiden – MTHFR polymorphisms – COC

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Introduction There are many cardiovascular problems occurring on the basis of thrombophilia which is characterized by disrupted balance of haemostatic mechanism. These problems results from interaction of multiple genetic and environmental factors. Single nucleotide polymorphism G1691A in factor V gene,

predicting the replacement of Arg506Gln, impairs the proteolytic degradation of factor Va and is associated with a risk of thrombotic events. MTHFR (5, 10-methylenetetrahydrofolate reductase) is the pivotal enzyme in folate metabolism and due to this also in many other metabolic pathways. Single nucleotide polymorphisms C677T and A1298C in the MTHFR gene results in decreased activity of this enzyme. Clinical implication of these mutations in MTHFR gene is hyperhomocysteinemia which is considered to be an independent risk factor for cardiovascular diseases. All these mutations are enhanced by using of combined oral contraception with a synergic risk for development of cardiovascular diseases.

Methods The first step of experimental part was genotypization, using PCR-restriction enzyme analysis (PCR-REA) and Real-time PCR. The second step was a statistical evaluation (χ^2 – chi square test of two variables, Unistat 5.1). Thirty-nine participants, including woman only, were tested. They were divided on the basis of personal and familial anamnesis into the groups with/without cardiovascular problems and furthermore to the subgroups depending on using combined oral contraception. All these information were obtained from questionnaire.

Results and Conclusion There was identified a significant association between presence of cardiovascular problems and polymorphisms MTHFR C677T (Figure 1) and then also sum of all non-standard alleles (Figure 2). This significant

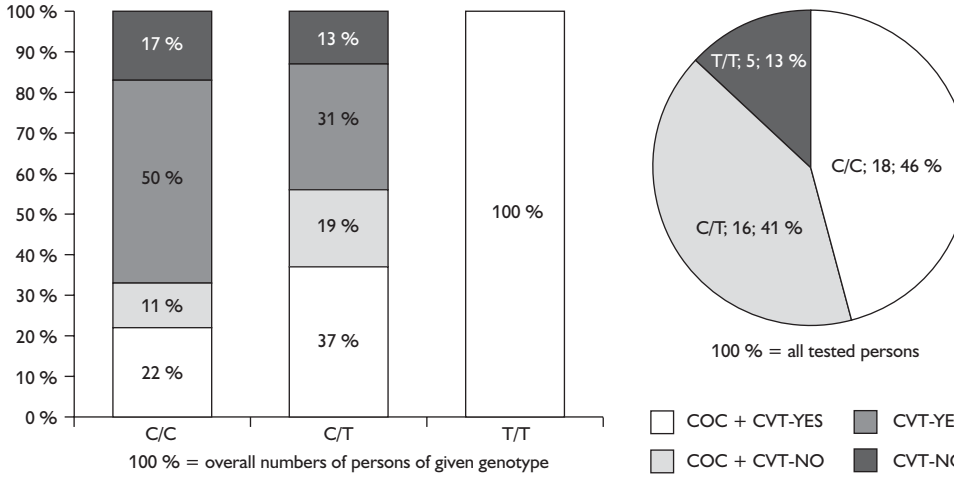

Figure 1 – Occurrence of cardiovascular troubles in dependence on COC-using and genotype of polymorphism C677T in MTHFR gene. CVT = cardiovascular troubles; COC = combined oral contraceptives

association was confirmed for the group of COC users. There were no non-standard homozygotes for FV Leiden and only two for polymorphism MTHFR A1298C in our tested group. No significant association was found for these two polymorphisms.

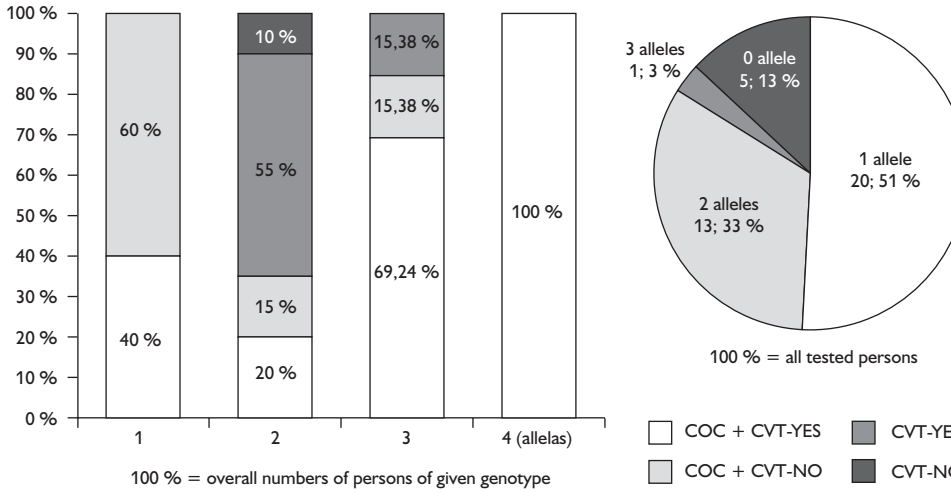

Figure 2 – Occurrence of cardiovascular troubles in dependence on COC-using and the number of non-standard alleles in tested genes. CVT = cardiovascular troubles; COC = combined oral contraceptives

Antiproliferative Activity of Methoxyspirobrassinol and its Synthetic Derivates

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Key words: Methoxyspirobrassinol – Antiproliferative – Iindole phytoalexins

This project was supported by the Slovak Research and Development Agency under the contract No. APVV-0514-06.

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Introduction Phytoalexins are produced by plants after exposure to physical, biological or chemical stress and a specific group of these metabolites represent indole phytoalexins produced by important plants of the family Cruciferae. Several indole phytoalexins (i.e. brassinin, spirobrassinin, brassilexin, camalexin, 1-methoxyspirobrassinin, 1-methoxyspirobrassinol and methoxyspirobrassinol methyl ether) have been found to possess significant antiproliferative activity against various cancer cells and this activity is supposed to be associated with the modulation of activity of transcription factors regulating cell cycle, differentiation and apoptosis. With respect to the epidemiologically proven cancer chemopreventive properties of brassica vegetables, antiproliferative effect of methoxyspirobrassinol and its synthetic derivates was studied to find out possible relationship between structure and antiproliferative effects on various human cancer cell lines.

Methods *Tested compounds*: (±)-1-methoxyspirobrassinol (2), cis-(±)-1-methoxyspirobrassinol methyl ether (3), trans-(±)-(1-tert-butoxycarbonyl)-2-hydroxy-2'-phenylaminospiro{indoline-3,5'-[4',5']dihydrothiazole} (K11), trans-(±)-(1-tert-butoxycarbonyl)-2-hydroxy-2'-(N-metylphenylamino-spiro{indoline-3,5'-[4',5']dihydrothiazole} (K12), cis-(±)-(1-tert-butoxykarbonyl)-2-hydroxy-2'-(N-metylphenylamino)-spiro-{indoline-3,5'-[4',5']-dihydrothiazole} (K13), (±)-1-acetylspirobrassinol (K109), (±)-1-benzoylspirobrassinol (K110), trans-(±)-(1-methoxy)-spirobrassinol phenyl ether (K131), cis-(±)-(1-methoxy)spirobrassinol phenyl ether (K132), (2R,3R)-1-methoxyspirobrassinol (1S,2R,5S)-menthyl ether (K133), (2S,3R)-1-methoxyspirobrassinol (1R,2S,5R)-menthyl ether (K134)

Human cancer cell lines: Jurkat – acute T-lymphoblastic leukemia, *MCF-7* – mammary gland adenocarcinoma.

Antiproliferative effect of the tested compounds was studied by using colorimetric microculture assay with the MTT end-point. The amount of MTT reduced to formazan is proportional to the number of viable cells. After 72 hours incubation the absorbance was measured at 540 nm using the automated MRX microplate reader (Dynatech laboratories UK). Absorbance of control wells was taken as 100%, and the results were expressed as a percent of control. Obtained data were converted into the IC_{50}.

Results and Conclusions Our data has shown that compound K12 possesses the highest antiproliferative activity with $IC_{50} = 5.3$ (μmol.l^{-1}) on Jurkat and $IC_{50} = 3.7$ (μmol.l^{-1}) on HeLa cells (Table 1). The binding of N-metylfenylamino group has significantly enhanced cytotoxic effect of trans form (K12), not of cis form (K13).

These results suggest the valid interest in structure and antiproliferative activitity relationship in searching for anticancer agent. Further studies are necesary to

investigate the mechanism of action and to find out the relationship between structure, character and position of substituents and their antiproliferative activity.

Table 1 – IC_{50} (μmol.l^{-1})

	2	3	K11	K12	K13	K109	K110	K131	K132	K133	K134
Jurkat	>100	44	47.7	**5.3**	>100	>100	50	100	100	54	58.0
MCF-7	>100	65	27.4	**3.7**	>100	>100	>100	100	100	84	70.5

Unconventional Sources of Antitussive Active Polysaccharides

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Key words: Polysaccharides – Antitussive activity – Malian medicinal plants

Presented works were supported by grants VEGA No. 1/3375/06 and APVV-0030-07.

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Introduction The plant polysaccharides have been reported to possess various biological effects including antitussive activity followed also during our previous experimental works. Furthermore, these substances are able to reduce cough comparably with commonly used antitussives without such severe side effects observed very often during most active antitussives codeine type treatment.

Presented experiments were aimed on effect of the subsequent unconventional herbal polysaccharide constituents on the citric acid-induced cough reflex and reactivity of airways smooth muscle *in vivo* conditions: polysaccharides isolated from leaves of *Trichilia emetica (TE)* and *Opilia celtidifolia (OC)*, and from fruit of *Crossopteryx febrifuga (CF),* the Malian medicinal plants. They are often uses in Mali as a remedy for wound healing, traditionally to relieve symptoms of respiratory infections and asthma, for cirrhosis treatment or malaria, tropical dysentery and hypertension.

For comparative purposes the most active in clinical practice used codeine as well as solvent (water for injection) used in experiments were tested under same conditions.

Methods All used substances were applied by per oral route of administration, plant carbohydrates in the dose 50 mg.kg^{-1} b.w., codeine in the dose 10 mg.kg^{-1} b.w. and solvent in the dose 1 ml.kg^{-1} b.w.

Antitussive activity of the herbal polysaccharides was tested on awaken male Trik strain guinea pigs weighing 200–350 g by chemical stimulation. Animals were located in a bodyplethysmograph box and were exposed to citric acid in concentration 0.3 M for 3 min interval, in which number of cough efforts was counted. The cough effort was defined as sudden enhancement expiratory flow associated with typical cough motion and sound followed by trained observer.

The reactivity of airways smooth muscles was expressed as values of specific airway resistance calculated by time difference between nasal and chest parts of bodyplethysmograph during normal breathing pattern.

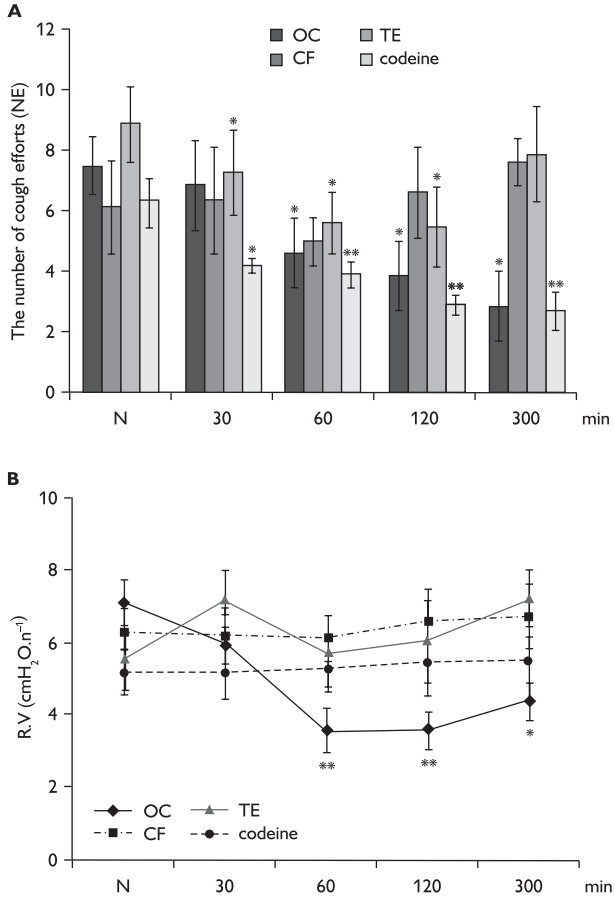

Figure 1 – The influence of the herbal polysaccharides on defence reflexes of the airways: ***A.*** *Modulation of citric acid-induced cough.* ***B.*** *Changes of specific airway resistance Both parameters registered before application (N) and after that in described time intervals (30, 60, 120, 300 min). Influence of polysaccharides on experimentally induced cough (A) compared with codeine and changes of specific airway resistance (B) confronted with solvent effect. (*p< 0.05 vs. N)*

Both, influence on citric acid-induced cough and specific airway resistance, were registered before any agent application and after that in 30, 60, 120 and 300 min time intervals. The minimal time interval between two measurements was 2 hours to prevent cough receptors adaptation as well as adaptation of laboratory animals on irritation.

All obtained data were statistically evaluated using Student t-test. A level of probability 0.05 and less were considered as significant.

Results and Conclusions Our experiments showed ability of herbal polysaccharides isolated from leaves *Trichilia emetica* and *Opilia celtidifolia* to reduce number of citric acid induced cough efforts. Prompt onset and shorter duration of the effect was recorded on *TE* administration, while slower onset and prolonged duration was registered on *OC* application. However, antitussive activity of these polysaccharides did not achieve the effect of codeine (Figure 1A). Furthermore, *OC* decreased values of specific airway resistance, sensitive predictor of airway smooth muscle reactivity *in vivo* conditions (Figure 2B). This bronchodilatory effect may participate on achieved cough suppression, either.

We did not found relationship between polysaccharides of *Crossopteryx febrifuga* and defense reflexes of the airways.

Drug Use in Patients with Oral Lichen Planus

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Key words: Oral lichen planus – Adverse drug reactions – Drug utilization

This study was supported by grants MSM ČR No. 0021620820 and 0021620849.

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Introduction Oral lichen planus (OLP) is a mucousal lesion of unknown ethiology. Among other factors like age, gender, immunopathological reactions, contact with restoration dental materials like amalgam, hepatitis C-associated

diseases and other systemic diseases and also drug-induced reactions are involved in its pathophysiological process. The aim of this study was to mark the difference of medication between patients with OLP or lichenoid lesions and controls.

Methods The study group consisted of 46 patients (34 women, 12 men, all aged 34–84 years) and the control group of 60 persons (43 women, 17 men). The patients with histopathologically proven OLP and the control subjects matched by age and sex without any signs of mucousal disorders were enrolled into the study. The evaluation of drug consumption was based on the Anatomical Therapeutic and Chemical (ATC) classification system.

Results and Conclusions The drug utilization in the OLP patients was significantly higher then that in the persons of the control group. On average the patients and the control persons consumed 4.5 and 3.1 of the prescribed medicaments per day and person, respectively. Approximately 93% of the patients (43 of 46) reported the daily intake of at least one or more of the drugs while only 68% of control subjects (41 of 60) were treated regularly. Table 1 summarizes the main ATC group representing the 90% drug prescribing segment.

Although the OLP ethiopathogenesis is complex and poorly understood, some interactions with the drug usage, the environmental and the lifestyle factors are reported. In comparison to the control group, the OLP patients in this study consumed a significantly higher number of daily medications, especially of those from the ATC groups of hypolipidemics, antidiabetics, nonsteroidal anti-inflammatory drugs and anxiolytics.

Table 1 – The 90% segment of drug prescribing in patients with oral lichen planus (OLP) and in control persons. A subject medicated with more than one drug will appear more than once according to the drugs consumed

	Number of persons (%)		
ATC group	OLP (n=46)	Controls (n=60)	χ^2p
C Cardiovascular system	31 (67.4)	17 (28.3)	0.0001
A Alimentary tract	22 (47.8)	9 (15.0)	0.0005
M Musculo-skeletal	13 (28.3)	7 (11.7)	0.0300
N Nervous system	13 (28.3)	0	–
B Blood	11 (23.9)	8 (13.3)	N.S.
H Systemic hormonal preparations	4 (8.7)	3 (5.0)	N.S.
R Respiratory system	4 (8.1)	0	–

 Prague Medical Report / Vol. 109 (2008) Suppl., p. 106–108

The Aromatase Inhibitors' Effect on Estrogen Synthesis *in situ* in the Model of Premenopausal Mammary Carcinogenesis

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Key words: Premenopausal mammary carcinogenesis – Aromatase inhibitors – Female rat

This project was supported by the grant number UK/66/2007, UK/67/2007 and ESF.

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Introduction Single-agent therapy with aromatase inhibitors has no established role in premenopausal breast cancer women. The results of some experimental works offer hypothesis that they would be suitable also for premenopausal women with breast cancer. In above cited experiments, the key role of i*n situ* estrogen synthesis via aromatase in the mammary gland tissue in the development and progression of breast cancer was proved. It is also supposed, that estrogen synthesized in situ has greater influence on the development of breast cancer than estrogen produced by ovaries**.** The efficacy and toxicity of aromatase inhibitors in the treatment of premenopausal women with breast cancer are also discussed among experimental and clinical oncologists.

The aim of this study was to create the experimental model of premenopausal mammary carcinogenesis and to investigate preventive effects of letrozole in it. We also wanted to evaluate the benefit of this therapy as well as the side effects on the organism.

Methods During our study 3 experiments were done. In every experiment, 60 intact female Sprague-Dawley rats 30–37 days old, weighing 130–180 grams were used. The animals were adapted to the standard condition of vivarium and were taken standard food for rats and water *ad libitum*. The rats were divided into 3 groups (20 animals in 1 group). Aromatase inhibitors (letrozole, exemestane and anastrozole) were used as chemo preventive agents. Chemoprevention began 7 days before chemo carcinogen administration and lasted et the end of every

experiment. Group 1 – the control group had taken food without aromatase inhibitor and the groups 2 and 3 with aromatase inhibitor in various concentrations. The N-methyl-N-nitrosourea (NMU) as chemo carcinogen was used to induce mammary carcinogenesis. NMU was injected intraperitoneally during period of 40–60 postnatal days of rats. Our model mimicked situation in healthy, but from the point of view of the development of breast cancer, high-risk premenopausal women.

Once a week the rats were weighed and palpated. The body weight of animals was evaluated and the local mammary tumours were assessed in terms of their presence, number, place and size. During the experiments water and food intake were measured. At the end of the experiments, the animals were killed by quick decapitation. The blood was taken to examine biochemical parameters of plasma lipid metabolism and serum levels of sex hormones. Mammary tumours, uterus and vagina were excised. The uterus and vagina were weighed and together with mammary tumours were sent for histological analysis. The mammary tumours incidence was assessed by Mann-Whitney U-test, the other parameters by one-way variance analysis (ANOVA) or Kruskal-Wallis test.

Results and Conclusions In the experiment with letrozole, letrozole resulted in significant decrease of incidence of the mammary tumours in the group Letro 1 ($P<0.00002$) compared with control and in the group Letro 10 total suppression of mammary carcinogenesis was observed. The adverse effects of letrozole

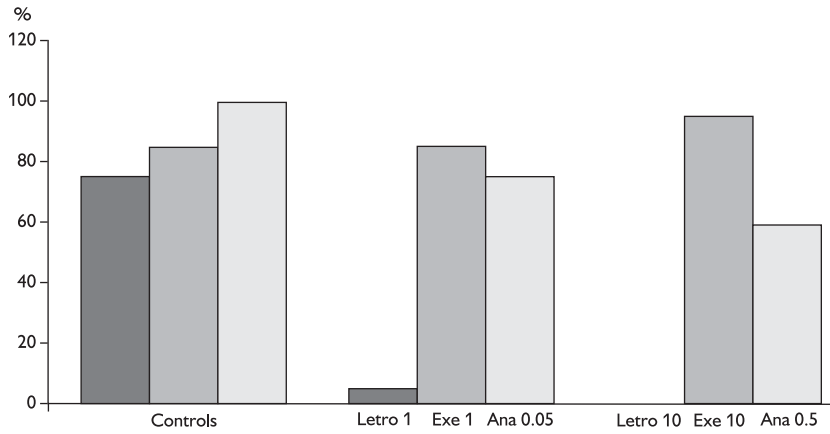

Figure 1 – Incidence of mammary tumours. Controls – control groups, Letro 1 – group with administered letrozole in concentration of 1 mg/kg in food, Letro 10 – group with administered letrozole in concentration of 10 mg/kg in food, Exe 1 – group with administered exemestane in concentration of 1 mg/kg in food, Exe 10 – group with administered exemestane in concentration of 10 mg/kg in food, Ana 0.05 – group with administered anastrozole in concentration of 0.05 mg/kg in food, Ana 0.5 – group with administered anastrozole in concentration of 0.5 mg/kg in food. Data are expressed as per cents. Significantly different, Controls vs Letro 1 $P<0.00002$, Controls vs Ana 0.5 $P<0.05$.

treatment were – the atrophic changes in the endometrium of uterus and tile-shaped epithelium in the vagina, significant increase in triacylglycerols, food intake and body weight gain. In the experiment with exemestane significant differences in the incidence of mammary tumours between treated and untreated animals were not observed. Exemestane significantly decreased parameters of lipid metabolism, significantly increased in food intake and body weight gain in the group Exe 10. In the experiment with anastrozole, anastrozole significantly suppressed tumour incidence in the group Ana 0.5 ($P<0.05$) in comparison with control group. A significant increase in body weight gain was found in the group Ana 0.5 compared with control (Figure 1).

Aromatase inhibitors are effective drugs for the therapy of postmenopausal women with a receptor-positive breast cancer. In our model of premenopausal mammary carcinogenesis tumour suppressive effect of non-steroidal aromatase inhibitors – letrozole and anastrozole was proved. Steroidal aromatase inhibitor – exemestane did not have tumour suppressive effect in our model of premenopausal mammary carcinogenesis. Present side effects arise from the suppression of estrogen synthesis and are typical for aromatase inhibitors treatment in postmenopausal women with breast cancer.

Human Metabolomic Biomarkers for PPARα Activation

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Key words: Metabolomics – Peroxisome proliferator-activated receptor α – Ultra-performance liquid chromatography – Time-of-flight mass spectrometry – Human – Biomarkers

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Introduction Metabolomics offers the opportunity to perform drug metabolite identification in detail and to uncover small molecule biomarkers of disease. To date, most investigations have been performed in laboratory animals. The metabolomic study of human volunteers and patients poses additional demands, in particular, the outbred nature of human populations compared with rodent species and, importantly, widely differing environmental and dietary exposures, together with differences gut microbiota that occur within human populations. The task of uncovering human metabolomic biomarkers is thus a challenging one.

The peroxisome proliferator-activated receptor alpha (PPARα) belongs to the nuclear receptor superfamily and plays a complex role in the control of lipid, amino acid and glucose homeostasis. In the clinic, PPARα agonist drugs are used in the treatment of disorders of lipid metabolism, such as hypercholesterolaemia. It would be advantageous to have readily measurable biomarkers of PPARα ligand effects in humans, as a window into the multiple metabolic effects that this group of drugs exerts.

Methods Towards this end, a study of 10 healthy volunteers was undertaken in Prague. Blood and urine samples were taken on days 0, 7 and 14 after the daily administration of a single 200 mg capsule of the PPARα agonist fenofibrate (Lipanthyl®). Serum and urine samples were shipped frozen on dry-ice to Bethesda, USA and metabolomic analysis performed using ultra-performance liquid chromatography-coupled time-of-flight mass spectrometry (UPLC-TOFMS). Resulting data matrices were combined with the clinical biochemistry data and were analysed using random forests machine learning algorithm.

Results Expected statistically significant decreases were observed in the serum concentrations of cholesterol, triglycerides and uric acid. Several additional biomarkers indicative of increased fatty acid β-oxidation were uncovered. These had not previously been reported in the mouse or rat. However, a single biomarker of enhanced NAD turnover, that had been previously reported in mice was found. One unexpected biomarker of gut microbiota metabolism was uncovered, different from the gut floral metabolite 2,8-dihydroxyquinoline and its β-D-glucuronide that were reported in mice treated with a PPARα agonist.

Conclusions This investigation demonstrates the proof of principle that human volunteers can be shown to exhibit statistically significantly different urinary excretion of metabolomic biomarkers of drug action. Biomarkers arising from the on-receptor (specific) and off-receptor (non-specific) effects of drugs in a clinical setting can thus be distinguished. Metabolomics offers promising new approaches to the direct investigation of the clinical pharmacology and toxicology of drugs.

The Use of ^{14}C Mannitol and Phenol Red in the Assessment of the Caco-2 Monolayer Integrity

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Key words: Caco-2 – Monolayer integrity – Phenol red – ^{14}C mannitol

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Introduction The human epithelial Caco-2 cell monolayer model has been widely used as a standard screening tool for studying the mechanism of drug transport. An assessment of monolayer integrity can be performed by measuring (i) transepithelial electrical resistance, (ii) permeability of hydrophilic paracellular markers, (iii) permeability of lucifer yellow or phenol red. We standardly use the assessing of the ^{14}C mannitol permeability for checking the monolayer integrity during the transport studies. We were looking for the method enabling the checking the integrity of each insert before the experiment. Phenol red (PR) seemed to be a good choice as a non-toxic, not metabolized or synthesized by cells compound and as a compound not influencing the cell growth and viability, and easy to be detected by spectrophotometry.

Methods Caco-2 cells (ECACC) were cultured in a standard manner. For transport studies, Caco-2 were seeded onto the Transwell inserts at a density of 2.5×10^5 cells/cm^2 and grown to late confluence (21–24 days).

Firstly, the possibility of usage of PR for assessing the monolayer integrity was checked: 500 μM PR was added to the apical compartment (n=72) for 1 hour incubation at 37°C and then the sample from the basolateral compartment was taken to count the permeability coefficient (Papp).

Secondly, the ^{14}C mannitol (n=11) study was done 2 days after the PR study (in the same direction with 2 hour incubation) to compare the results of the PR and the mannitol permeability.

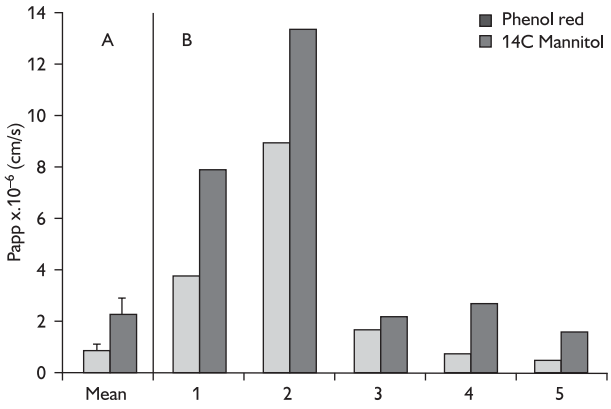

Figure 1 – The PR Papp (n=64) determinated 2 days before the assessment of ^{14}C mannitol permeability (n=11). A) Mean Papp ± SD. B) The choosen inserts (1–5).

Results After excluding 8 inserts with transported amount > 0.5 % (Papp PR > 1.8 cm/s), the PR Papp (n=64) was 0.82 ± 0.28 cm/s and the transported amount 0.23 ± 0.08 %. The Papp of mannitol (n=11) was 2.26 ± 0.62 cm/s and the percent transport 1.63 ± 0.44 (Figure 1).

Discussion The monolayers with the high Papp of PR also showed the high Papp of mannitol (Figure 1/B). For decreasing the influence of PR on cells 1 hour incubation was used (enough time to assess the integrity). The incubation time of mannitol is dependent on the time of transport studies, usually 2 hours.

Conclusion Phenol red is a suitable method for preliminary assessing the monolayer integrity before transport studies.

Synthesis a Novel Aryl-, N-2 Substituted Hexahydro-1*H*-pyrido[4,3-b]indole Derivatives and their α-adrenolytic Activity

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Key words: Stobadine – Methamphetamine – α-adrenolytic activity – Antioxidants – Pyridoindole

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Introduction It has been reported that Carbidine or their (-) enantiomeric form Stobadine (4a(R),9b(S)-(-)-cis-2,8-dimentyl-2,3,4,4a,5,9b-hexahydro-1*H*-pyrido [4,3-b]indole dihydrochloride) are effective free radical scavengers, antipsychotic and α-adrenolytic activity agents with thymoleptic properties. In animal studies not only depressed the central nervous system (CNS) but also enhanced the stereotyped behavior induced by methamphetamine, which is a characteristic property of thymoleptics. Hence, an attempt was made to prepare novel hexahydro-1*H*-pyrido[4,3-b]indole derivatives with potentiated antioxidant activity compared to Stobadine without α-adrenolytic activity, which is undesirable effect in the treatment of the heart attack and strokes or other neurodegenerative diseases, as well (Figure 1).

Methods The experiments were performed on male Wistar rats (250–270 g, Breeding of IEPh SAS Dobrá Voda, Slovak Republic). The rats were sacrificed by cervical dislocations, the thoracic aorta was removed, cleaned from adherent tissue in physiological salt solution (PSS), and cut into 8 rings, each approximately 2 to 3 mm long. Special care was taken not to damage the endothelium. The rings were mounted between two L-shaped hooks in water-jacketed (37° ± 0.5°C) chambers containing PSS bubbled with 95% O_2 and 5% CO_2 at pH 7.4. The composition of PSS was (in mmol/l): NaCl (118.0), KCl (4.7), KH_2PO_4 (1.2), $MgSO_4$ (1.2), $CaCl_2$ (2.5), $NaHCO_3$ (25.0) and glucose (11.0). The preparations were connected to an isometric transducer (M 1101, Czech Republic) and stretched passively to optimal length by imposing an optimal initial tension of 20 mN, as tested in preliminary experiments. After application of the initial tension, the arterial preparations were equilibrated for 60 minutes. Isometric contractions were recorded on a Kutesz 185 line-recorder (Hungary). The experimental protocols was as follows: Preparations were contracted by phenylephrine in concentrations increasing cumulatively from 10^{-9} to 10^{-6} mol/l. Subsequently, the preparations were washed several times with

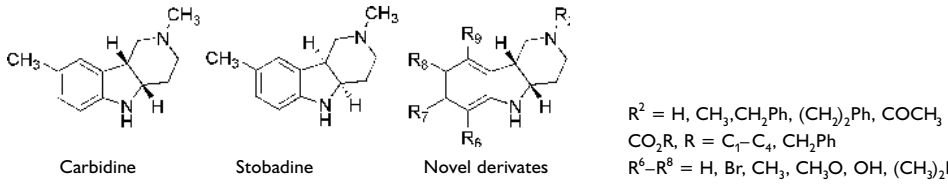

Figure 1 – The structure of Carbidine, Stobadine and novel synthetized derivatives of hexahydro-γ-carboline.

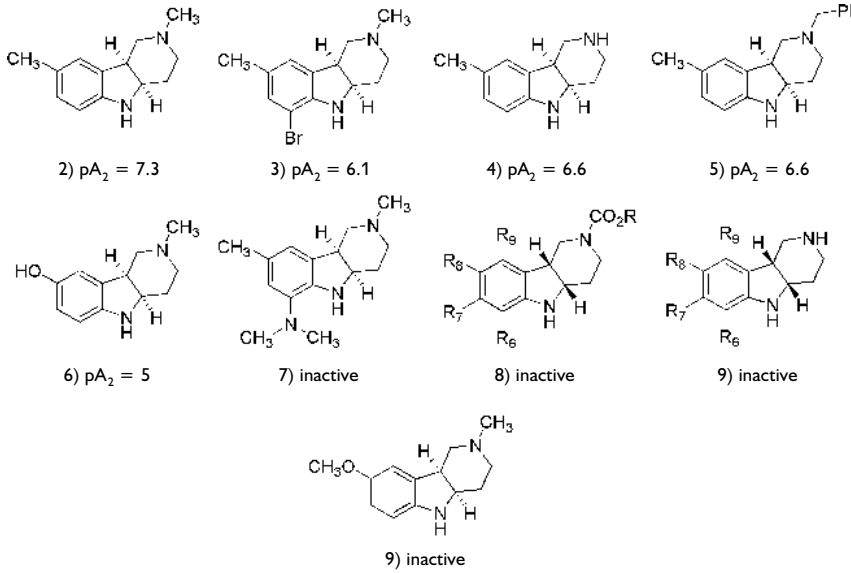

Figure 2 – α-adrenolytic activity structure-relationship.

PSS and the relaxed to initial tension values. After 60 minutes, the concentration-response curve of phenylephrine was performed again, and the second curve was considered to be the control. Then the preparations were washed several times during the 30-minute period. The drug was then tested, in the concentration of 10^{-7} mol/l, added to the PSS for 30 minutes, and the concentration-response curve of phenylephrine was done. This protocol was repeated with the compound at concentrations 10^{-6} and 10^{-5} mol/l. Four compounds were tested in parallel, each in a separate preparation vessel. Sensitivity of the preparations to phenylephrine was expressed from the concentration-response curve, the maximal response in control conditions was considered as 100%. The EC_{50} was evaluated as the concentration of phenylephrine when the responses reached 50% of the maximal values, α-adrenolytic activity of the compounds was expressed in pA_2 (negative logarithms of the antagonist concentration inducting depression of the effect of the agonist by 50%).

Results and Conclusion Stobadine (2) is a competitive antagonist of the α-adrenolytic receptors. Its 6-bromo (3), 2-unsubstituted (4), 2-benzyl (5) and 8-hydroxyderivative (6) had a similar effect. Derivatization of Stobadine with $(CH_3)_2N$ group in the position 6- (7), or 2,3,4,4a,5,9b-hexahydro-1*H*-pyrido [4,3-b]indole skeleton with alkoxycarbonyl fragment on position 2-(8), or other 2-unsubstituted molecules (9) showed to be an advantage from the point of view of elimination of undesirable α-adrenolytic activity. Modification of Stobadine in the position 8- and replacement of the CH_3 group with the CH_3O function (10) had conformable effect. For most tested substances, α-adrenolytic activity was eliminated. Only seven out of all substances studied exerted a mild to moderate α-adrenolytic activity (Figure 2).

Effect of Ω-3 Fatty Acids on the Aortic Function of Lewis and Spontaneously Hypertensive Rats

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Key words: PUFA – NOS activity – Rat aorta – Endothelium – Spontaneously hypertensive rats

This work was supported by grant APVV-51-059505 and partially by VEGA, 2/0086/08 and 2/7064/07.

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Introduction Results of clinical trials and experimental studies indicate that consumption of Ω-3 polyunsaturated fatty acids (PUFA) is associated with decreased risk for cardiovascular diseases. Besides their antidysrhythmic properties, protective effects of PUFA are attributable to their effects on vascular smooth muscle cell and endothelial cell function. The aim of the study was to evaluate the influence of PUFA consumption on the endothelial function of the aorta of Lewis (LEW) and spontaneously hypertensive rats (SHR).

Methods Experiments were performed on 1-year-old SHR and age-matched LEW rats as controls. Both groups were divided into treated with PUFA (Vesteralens, Norway, 30 mg/day, for 2 months) and untreated rats. The thoracic aorta was excised from anaesthetised animals and used for NO-synthase (NOS) activity measurement and functional studies. NOS activity was measured in the homogenates of the aorta by determination of [^{3}H]-L-citrulline formation from [^{3}H]-L-arginine. Functional vessel studies were performed under isometric conditions on rings of aorta. The rings were precontracted with 1 μmol/l phenylephrine (PE) and relaxant responses to acetylcholine (ACh, 0.01–100 μmol/l) were tested at the plateau of the contraction.

The contractile responses to phenylephrine (PE, 1 μmol/l) before and after inhibition of NOS with 100 μmol/l Nω-nitro-L-arginine methyl ester (L-NAME) were evaluated.

Results and Conclusions Basal NOS activity in the aorta of SHR was significantly lower (1.10 ± 0.22 pmol/min/mg) than in LEW (6.30 ± 0.29 pmol/min/mg). PUFA treatment led to the increase of NOS activity in the aorta of SHR and LEW (1.89+0.26 and 7.36+0.11 pmol/mg/min, respectively). The endothelium-dependent relaxation of the aorta was diminished in SHR compared to LEW. It was manifested by decreased responses of preparations to ACh – the maximal relaxation achieved 64% compared to 34% of contraction found in the aortas of LEW. Administration of PUFA tended to improve the endothelium-dependent relaxation of the aorta of SHR and LEW.

PE-induced contractions of the aortas were not different among the groups. After L-NAME, contractile responses of the LEW aortas to PE were potentiated compared to control responses ($P<0.01$) suggesting a high NO tone in these vessels. Unlike in SHR aortas, where PE-induced contraction was not changed by NOS inhibition, basal tone of NO seems to be low. Administration of PUFA did not significantly increase the NOS-independent PE-induced contraction either of LEW or SHR aortas, in spite of increased NOS activity. This

effect might be a consequence of the inhibitory effect of PUFA on intracellular Ca^{2+} release and Ca^{2+} channels in vascular smooth muscle leading to NO-independent relaxation.

Our results suggest that PUFA supplementation may improve aortic function by both NO-dependent and NO-independent mechanisms.

Enhancement of Analgesic Effect of Paracetamol and Ibuprofen with Rilmenidine in Mice

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Key words: Rilmenidine – Paracetamol – Ibuprofen – Antinociception – Writihing test

This project was supported by the research grants VS MSM ČR No. 0021620816 and IGA MZ ČR NR/9072-3.

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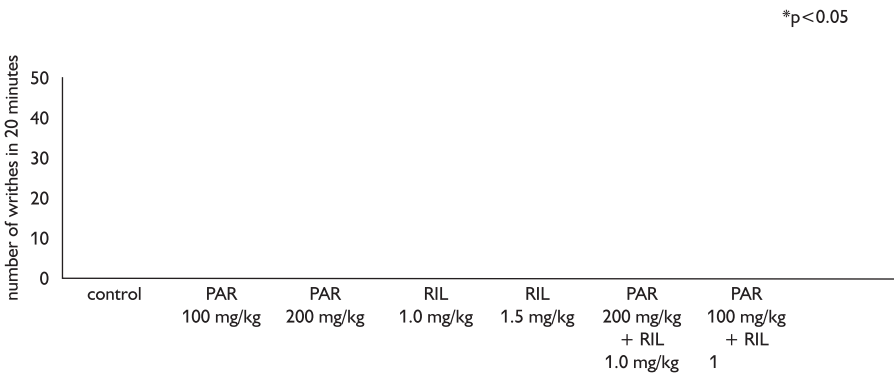
Introduction The aim of the study was to investigate the antinociceptive effect of rilmenidine, an imidazoline (I1) agonist, in the writhing test in mice. Subsequently we have explored the influence of rilmenidine on analgesic action of paracetamol and a nonsteroidal anti-inflammatory drug ibuprofen. Experimental studies of combination of these agents have not yet been published.

Methods Male NMRI mice were used. For the writhing test, acetic acid (0.7%, 0.1 ml/10 g) was injected into the peritoneal cavity. The number of writhes was counted during a 20 min period after the administration of acetic acid solution. A writhe was defined as a contraction of the abdominal muscles accompanied by an elongation of the body and extension of hind limbs. Dose-response curves were constructed in order to assess the antinociceptive action of paracetamol, ibuprofen rilmenidine and paracetamol-rilmenidine mixture given orally. The interaction of drugs was investigated by a fixed-dose analysis or an isobolographic analysis (the method based on comparisons of doses that are determined to be equieffective). In our study, rilmenidine and paracetamol were simultaneously coadministered orally at doses of the ED-50 values and fractions (1/2, 1/4, and 1/8) of the ED-50 of each drug. From the dose-response curves of the combined drugs, the ED-50 value of the mixture was calculated, and this dose combination was used for

plotting the isobologram. To calculate the ED-50 values of each drug for isobolographic analysis, the total number of writhes in 20 minutes was converted to a percentage of control according to the formula: % maximal possible effect = (mean ± SEM of control) – (mean ± SEM of treated group)/ (mean ± SEM of control).

Results and Conclusions There was a significant dose-related antinociceptive effect of rilmenidine in the writhing test with ED-50 value at 2.46 mg/kg and a



dose-related antinociceptive effect of paracetamol with ED-50 value at 214.80 mg/kg. A fixed-dose analysis showed that rilmenidine in the 1.5 mg/kg dose significantly ($p < 0.05$, examined by one-way ANOVA) potentiated the antinociceptive effect of paracetamol at the 200 mg/kg dose, but not the analgesic effect of ibuprofen in the 10 mg/kg or 30 mg/kg dose. An isobolographic analysis evaluating the interaction of simultaneous administration of fixed ratios of rilmenidine with paracetamol revealed a synergistic interaction.

In conclusion, the results indicate that the analgesic action of paracetamol is enhanced by rilmenidine in acute visceral pain test. The interaction between paracetamol and rilmenidine may involve supraspinal mechanisms. We have not found evidence for a synergistic interaction between systemic nonsteroidal anti-inflammatory drug ibuprofen and rilmenidine.

Evidence of Cross-talk between Aryl Hydrocarbon Receptor and Glucocorticoid Receptor in Placental Trophoblast JEG3 Cells

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Key words: Glucocorticoids – Arylhydrocarbon receptor – Dioxin – Cross-talk

This project was supported by the grant number GA ČR 303/07/0128 (P.P.).

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Introduction Aryl Hydrocarbon Receptor (AhR) and glucocorticoid receptor (GR) play crucial role in regulation of drug metabolizing enzymes and in many essentials physiological processes. CYP1A1, one isoform of cytochrome P450 also known as Aryl Hydrocarbon Hydroxylase (AHH), is important biotransformation enzyme which metabolizes several drugs widely used in pharmacotherapy. On the other side this enzyme plays a key role in bioactivation of procarcinogens and proteratogens such as polycyclic aromatic hydrocarbons (PAHs) to form DNA-adducts.

Expression of Cyp1a1 is transcriptionally regulated through ligand-activated Aryl hydrocarbon receptor. Glucocorticoids are reported to further augment the induction of CYP1A1 via activated AhR.

The aim of this work was to study the cross-talk between these two receptors in transactivation of CYP1A1 in placental JEG3 cell line exposed to the prototype AhR ligand 2,3,7,8-tetrachlorobenzo-p-dioxin (TCDD) alone and in combination with glucocorticoid drug dexamethasone.

Methods The effect of dexamethasone on TCDD-mediated transactivation was assessed employing real time-RT-PCR and expression of CYP1A1 mRNAs analyzed after 6–48 h exposition to these compounds. In addition, reporter assay in transiently transfected cells was used with two gene reporter plasmids containing promoter or responsive sequences of CYP1A1 gene. Single concentration of dexamethasone (50 nM) and TCDD (5 nM) was used in these experiments.

Results and Conclusions Our preliminary results show that co-treatment with dexamethasone caused elevation of TCDD-induced CYP1A1 mRNA in JEG3 cells. This effect was blocked by the GR antagonist RU486. On the other side 24-h treatment with dexamethasone did not increase transactivation of CYP1A1 gene in gene reporter assay.

Based on out preliminary data, we can suggest cross-talk of GR/AhR pathways in gene regulation of CYP1A1 in JEG3 cells, which is not likely at the level of transcriptional regulation.

Renal Accumulation of Radiolabeled Receptor-specific Peptides

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Key words: Membrane transport – Radiodiagnostics – Radiotherapy – Somatostatin

This project was supported by grant IGA MZ ČR NR/9208-3.

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Introduction Radiolabeled somatostatin receptor-specific peptides are useful in diagnosis or radiotherapy of somatostatin-positive neuroendocrine tumors.

However, significant renal uptake of the radiopeptides may result in radiotoxicological injure of the kidney. Multi-ligand endocytic megalin/cubilin receptor in renal proximal tubules might be the transport mechanism responsible for the retention. The aim of the study was to compare the renal accumulation of two somatostatin receptor-targeted peptides, DOTA-Tyr^3-octreotate labelled with ^{111}In (^{111}In-DOTA-TATE), and glucose-Tyr^3-octreotate labelled with ^{125}I (^{125}I-GLUC-TATE) in vitro and to analyze some factors affecting this process.

Methods The renal accumulation of the radiopeptides was determined using fresh renal cells isolated from rat kidneys. The cells were isolated by means of the two-phase collagenase perfusion method. The cell viability was determined using trypan blue exclusion method. Incubation with radiopeptides was carried out for 2–30 min at 37°C. After incubation, the cells were four times washed and separated by centrifugation. The radioactivity of the cell fractions was then measured. The influence of selected inhibitors of megalin active endocytosis (albumin, gentamicin) and the effect of inhibition of energy-dependent transport processes on the cellular uptake were also investigated.

Results and Conclusions ^{125}I-GLUC-TATE exerted significantly higher renal uptake in isolated renal rat cells in comparison with ^{111}In-DOTA-TATE (Table 1). The accumulation of the radiopeptides was time-dependent. The ^{111}In-DOTA-TATE renal uptake was partly inhibited during incubation with albumin or gentamicin. However, the inhibition potency of these compounds was unsatisfactory. The uptake was significantly decreased at low incubation temperature (Table 1). The results showed that the undesirable renal uptake was partly decreased by inhibitors of receptor-mediated endocytosis and by a block of energy-dependent processes. A significant participation of active transport processes in renal accumulation of the studied peptides should be considered. The megalin/cubilin receptor complex in renal tubules seems to be partly involved in this process.

Table 1 – Uptake of the radiopeptides in the isolated renal rat cells (% dose/10^6 cells)

	Incubation time (min)						
Compound	2	5	15	30	60	90	120
^{125}I-GLUC-TATE	1.00	1.23	1.71	1.81	2.28	2.21	3.46
^{111}In-DOTA-TATE	0.20	0.40	0.58	0.76	1.08	1.05	1.08
^{111}In-DOTA-TATE+albumin	0.16	0.28	0.55	0.45	0.53	0.60	0.64
^{111}In-DOTA-TATE+gentamicin	0.29	0.41	0.48	0.57	0.52	0.82	0.84
^{111}In-DOTA-TATE 2 °C	0.14	0.21	0.13	0.19	0.23	0.19	0.26

 Prague Medical Report / Vol. 109 (2008) Suppl., p. 120–121

Efect of D-penicillamine on Hyaluronan Degradation by Cupric Ions Plus Ascorbate

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Key words: Hyaluronan – D-penicillamine – Transition metal ions – NSAIDs – Reactive oxygen species

The VEGA grants 2/0003/08, 2/7033/27, and 2/7028/27) and APVV grants 51-033205, 51-017905, as well as APVT grants 99-P03305 and 20-0045/04) are gratefully acknowledged.

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Introduction Hyaluronan (HA) is a high-molar-mass linear polysaccharide present in many organs, especially in skin, vitreous humour, synovial fluid and cartilage. It is known as a scavenger of reactive oxygen species such as $^{\bullet}OH$ radicals. On the other hand, HA is readily degraded by reactive oxygen species (ROS) in inflammatory joint diseases, which is accompanied by production of low-molar-mass HA fragments. D-penicilamine is a β-merkaptovaline; it functions as an immunomodulating, third-line disease-modifying anti-rheumatoid drug in treatment of severe rheumatoid arthritis

The aim of the study was to test the ability of D-penicillamine to act as a pro-oxidant or an antioxidant in the system composed of hyaluronan, cupric ions in the presence or absence of ascorbate. Production of free radicals was demonstrated by two methodologies.

Methods Five hyaluronan samples of various weight-molar-mass were exposed to oxidative degradation by the reaction systems: i) ascorbate and cupric ions, ii) D-penicillamine and cupric ions, iii) cupric ions, D-penicillamine and ascorbate. The kinetics of hyaluronan degradation was monitored by rotational viscometry. To identify the generated ROS in a selected sample, EPR spectroscopy was applied.

Results and Conclusion Viscosity values of the HA sample solutions degraded by the system ascorbate and cupric ions gradually decreased. However, testing

D-penicillamine as a reducing agent of cupric ions revealed no degradation of any HA samples. Addition of various drug concentrations inhibited viscosity decline to a certain time period followed by much steeper degradation. EPR spectroscopy demonstrated the generation of $^{\bullet}OH$ radicals even in presence of copper ions and the drug, elimination of free radicals by ascorbate and their gradual regeneration.

Based on the results we can conclude that on applying rotational viscometry and EPR spectroscopy, initial anti-oxidative action of D-penicillamine followed by extensive pro-oxidative conditions were determined. The latter situation may be beneficial in the treatment of rheumatoid arthritis since generated hydroxyl radicals are responsible for the decomposition of metaloproteinases, which are supposed to damage joint cartilage in severe rheumatoid arthritis. In addition to hydroxyl radicals, thyil and disulfide radicals, several further radicals may be implicated in these reaction systems.

Cholesterol and Cytochrome P450 4A

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Key words: Cholesterol – Cytochrome P450 4A – Diet

This project was covered by the MSM ČR grants 1P05OC065 (COST B25.003) and No. 6198959216.

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Introduction In healthy rats, high cholesterol diet leads to an increase of triacylglycerol content in the liver tissue. This increase is caused by induced synthesis of fatty acids and reduced catabolism of free fatty acids by ω-oxidation. Such data from literature indicate that organisms need to maintain a certain cholesterol/triacylglycerol balance. The β-oxidation (pathway in fatty acids metabolism) is associated with members of the CYP450 4A gene family – CYP4A1 and CYP4A2. In this work, the possible effect of cholesterol excess on β-oxidation in microsomes was studied.

Methods Male Wistar SPF rats (b.w. 180–220 g) were fed ad libitum on standard laboratory diet (STD, KrmiMo Mohelsky, Brno, Czech Republic) or on a high cholesterol diet (HCD, prepared by adding 1% [w/w] cholesterol to the standard diet) for 3 weeks. The hepatic expression of the CYP4A isoforms 4A1 and 4A2

was determined by real-time PCR using SYBR Green PCR Master Mix in an Abi Prism 7700 Sequence Detection system. All experiments with animals were approved by the Ethics Committee, Ministry of Education, Czech Republic.

Results and Conclusions Feeding healthy rats with high cholesterol diet (ad libitum, 21 days) caused a significant decrease of both CYP450 4A1 and CYP450 4A2 mRNA levels (1.5 and 1.8 fold respectively; standard *vs* high cholesterol diet).

Levels of CYP4A1 and CYP4A2 mRNA were down-regulated by HCD itself. It is very noteworthy that cholesterol itself influences the expression of enzymes of the cytochrome P450 4A family. A possible explanation of this observation may be based on the fact, that liver cells are (due to presence of abundant cholesterol) forced to synthesize fatty acids to keep the cholesterol-fatty acid balance. As CYP4A enzymes take part in fatty acid metabolism, their expression should be down-regulated to maintain the appropriate levels of fatty acids in the liver. However, the precise mechanism of this cholesterol action remains unknown and should be elucidated in the next studies.

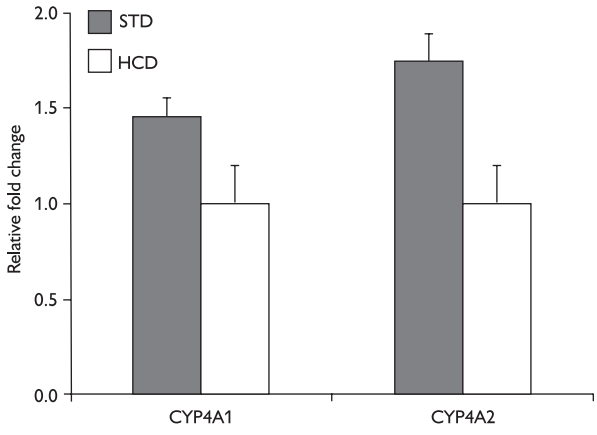

Figure 1 – Expression of CYP4A1 mRNA and CYP4A2 mRNA in rats fed on experimental diets. STD-standard diet, HCD-high-cholesterol diet. Data are derived from 7 animals, $p < 0.02$.

The Role of Bilirubin and UGT1A1 Mutations in Health and Disease

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Key words: Bilirubin – UGT1A1 mutations – Oxidative stress

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Introduction Bilirubin, the principle bile pigment, is the end product of heme catabolic pathway in the systemic circulation. For many years, bilirubin was thought to have no physiological function other than that of a waste product of heme catabolism – useless at best and toxic at worst. Although hyperbilirubinemia in neonates has been shown to be toxic for central nervous system, studies performed during the past two decades have found that bilirubin has a number of beneficiary effects. In addition, there is now a strong body of evidence suggesting that bilirubin may have a protective role in prevention of a number of diseases including atherosclerosis and cancer, as well as certain inflammatory, autoimmune and degenerative diseases. The serum level of bilirubin is regulated by the activity of hepatic bilirubin UDP-glucuronosyl transferase, encoded by the UGT1A1 gene, which is responsible for bilirubin conjugation with glucuronic acid in the liver and its elimination of bilirubin via biliary system into gastrointestinal tract. The homozygous state for the frequent variant $A(TA)_7TAA$ (known also as UGT1A1*28) allele of the TATA-box in the UGT1A1 promoter reduces the transcription activity of the gene and decreases hepatic UGT1A1 enzyme activity to about 30% of normal levels. This mutation is responsible for the manifestation of benign hyperbilirubinemia (known also as Gilbert syndrome) in the majority of affected Caucasians. Gilbert syndrome is a common autosomal recessive condition, characterized by mild, chronic, non-hemolytic unconjugated hyperbilirubinemia in the absence of liver disease.

Aims To analyze available data on the impact of both elevated serum bilirubin levels and UGT1A1 mutations on human health.

Results Serum bilirubin within a physiological range has been inversely related to cardiovascular disease in retrospective, prospective and meta-analytic studies. The same inverse relationship is true also for mildly elevated serum levels of unconjugated bilirubin characteristic for Gilbert syndrome. In fact, mild unconjugated hyperbilirubinemia due to UGT1A1*28 allele homozygosity has been associated with decreased incidence of coronary heart disease, certain types of cancer as well as other oxidative stress diseases such as schizophrenia. On the other hand, subjects with Gilbert syndrome are at higher risk of development of severe side effects of certain drugs such as inhibitors of topoisomerase or certain antiviral drugs, which are biotransformed in the liver tissue also by UGT1A1. Due to this fact, patients requiring such therapy should undergo UGT1A1 genotyping prior initiation of treatment with these drugs.

Conclusions Serum bilirubin levels as well as UGT1A1 status are important predictors of oxidative stress-mediated diseases as well as possible cytotoxicity of certain drugs. Therefore, both parameters seem to be increasingly important in predicting these pathophysiological consequences.

Comparison of Vessel Segments Reactivity of Rabbit: Ear Artery vs. Mesenteric Arteries

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Key words: Vessel reactivity – Rabbit – Ear artery – Perfusion

This project was supported by the grant VEGA number 1/2293/05.

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Introduction Vascular bed of rabbit a. auricularis (ear artery) is an important area of regulation of body temperature for these rodents. Main signal pathway of vasoconstriction or vasodilation is mediated by release of noradrenaline, respectively acetylcholine from nerve endings. The same regulation controls vessel tone of a. mesenterica superior and inferior as characteristic vessel beds of splanchnic area. Compound L-NAME (N-nitro-L-arginine methylester) acts as a false precursor of NO synthesis and thus reduces vasodilative and enhances vasoconstrictive responses.

The aim of our *in vitro* study was to verify and assess expected much stronger reactivity of ear arterial segments than mesenteric arterial segments to basic vegetative mediators with and without presence of L-NAME.

Methods Male New Zealand white rabbits were sacrificed and segments of following arteries were excised: a. auricularis, a. mesenterica superior and a. mesenterica inferior. Vessel preparations were cut to the length of 10–12 mm, bathed and tested in vitro by perfusion method. Vessel segments were perfused in freshly prepared Tyrode's solution and subjected to applications of consecutively increasing bolus doses of noradrenaline: 10, 50, 100, 500 ng, 1, 10 μg. After adding of L-NAME into the solution (concentration 10^{-5} M) the same order of vasoconstrictive stimuli followed. Thereafter, arterial segments were precontracted by incubation with stable concentration of noradrenaline (10^{-5} M) and their relaxations were tested by bolus dose of acetylcholine (20 μg).

Results and Conclusions Evaluated vessels exhibited very obvious multiplied differences in contraction amplitudes between ear arteries and both mesenteric arteries at each dose of noradrenaline without L-NAME in the solution and maintained clearly significant differences also with its presence. Relaxation responses of precontracted ear arteries were expressed by average value 15.1%, while those of mesenteric arteries by value 6.1% and 5.2% (a. m. superior and a. m. inferior, respectively); differences also reached a significant level.

The results show that segments of rabbit ear arteries exert several times higher reactivity to basic vegetative mediators than typical splanchnic arterial segments not only at vasoconstrictive responses, but even at vasodilative responses in conditions of extensive inhibition of normal NO signaling.

The Mechanisms Regulating Cytochrome P450 1B1 Expression and their Importance for Metabolic Activation of Promutagens

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Key words: Aryl hydrocarbon receptor – CYP1B1 – Estrogen receptor – Polyaromatic compounds – Cytokines

This project was supported by the grant number GA ČR 524/069/0517.

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Introduction Although the aryl hydrocarbon receptor (AhR) plays a major role in regulation of CYP1B1 expression, other important regulators are also involved, including estrogen receptors (ER), cyclic AMP-response element-binding protein (CREB), or AP1 and Sp1 transcription factors. Thus, CYP1B1 levels are regulated through an interaction of a number of factors, which may determine its tissue specific expression and/or induction. Wide distribution of CYP1B1 and its involvement in biotransformation of xenobiotics, as well as of endogenous substrates, indicate its significant role in tumorigenesis and in hormone metabolism. This presentation will briefly summarize current understanding of CYP1B1 regulation and our results regarding the interactions of pro-inflammatory

cytokine TNF-a and AhR in regulation of CYP1B1 and formation of genotoxic metabolites of polyaromatic compounds.

Methods We analyzed impact of model CYP1B1 inducers using Western blotting and real-time RT-PCR. We also employed model chemical inhibitors and siRNA-mediated gene knock-down, in order to understand possible contribution of individual signaling pathways to CYP1B1 up-regulation.

Results and Conclusions TNF-α can disrupt the balance of benzo[a]pyrene (BaP)-induced CYP1 expression in rat liver epithelial cells, leading to an enhanced formation of BPDE-DNA adducts, as well as the amplification of further effects associated with the genotoxicity of BaP. Our results seem to indicate that inflammatory conditions might enhance genotoxic effects of carcinogenic polycyclic aromatic hydrocarbons through upregulation of CYP1B1 expression. Analysis of signaling pathways and transcription factors binding to enhancer/promoter region of CYP1B1 should provide necessary information for understanding of CYP1B1 under inflammatory conditions.

Behavioral Profile of Different α-2 Adrenoceptor Ligands in Mice

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Key words: Aggression – Animal models – Behavioural pharmacology – α-2 adrenoceptor

Supported by the grants IGA MZ ČR NR/9369-3 and the VZ 0021620816.

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Introduction: The aim of the present study was to ascertain behavioral profile of α-2 adrenoceptor (α_2-AR) agonist dexmedetomidine (DEX), partial agonist naphtylmedetomidine (NFT) and antagonist atipamezole (ATI) in mice.

Methods Behavioral effects were studied in the activity cage and in the social conflict test in male mice (after three weeks of individual housing) in aggressive and sociable mice.

Results and Conclusions DXM (5–40 μg/kg i.p.) decreased locomotion in the activity cage (ED_{50} [95% CI] 14.1 μg/kg [9.04–18.48]), but the drug did not reduce locomotion during social conflict. The only significant effect during social conflict was a selective and dose-dependent antiaggressive effect in aggressive mice (ED_{50} [95% CI] 13.48 μg/kg [7.72–22.63]) and a selective reduction of social investigation in sociable mice. NFT (150–1200 μg/kg i.p.) dose-dependently decreased locomotion in the activity cage (ED_{50} [95% CI] 664.08 μg/kg [460.71–1698.08]). The only significant effect during social conflict was a selective and dose dependent antiaggressive effect in aggressive mice observed already after the lowest dose (ED_{50} [95% CI] 126.26 μg/kg [41.70–187.72]), while the sociability and locomotion were attenuated only after the highest dose of NFT. ATI (0.1–10 mg/kg) had no effect on locomotion in the activity cage and did not influence behavior in the social conflict test in aggressive mice.

The present results suggest that drugs with agonistic activity at α_2-AR inhibit predominantly dominant behaviour evoked by biologically important stimuli. In a novel environment in the activity cage, we observed inhibition of locomotion and sedation. In sociable mice, the most prominent effect after DXM and NFT treatment was inhibition of sociable activities. In aggressive mice, we observed very potent and selective antiaggressive effect. The present study suggests promising role of partial α_2-AR agonists in the treatment of aggressive states.

Table 1 – The effect of DXM, NFT and ATI on the number of aggressive acts (attack, threat, tail rattle) in aggressive mice during the social conflict

DXM dose	Control	5 μg/kg	10 μg/kg	20 μg/kg
Mean (SEM)	161.23 (12.01)	126.4 (15.71)	108.89 (13.94)	53.57 (14.38)
NFT dose	Control	150 μg/kg	300 μg/kg	600 μg/kg
Mean (SEM)	56.8 (7.75)	25.95 (6.77)	10.65 (5.25)	0.0 (0.0)
ATI dose	Control	0.1 mg/kg	1 mg/kg	10 mg/kg
Mean (SEM)	48.45 (9.23)	25.45 (12.08)	62.55 (14.34)	71.09 (16.06)

Usage LC-MS Technique for Determination of Enalapril and its Metabolite Enalaprilat in Pharmacokinetic Studies

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Key words: ACE inhibitors – Enalapril – LC-MS method – Pharmacokinetics

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Introduction Enalapril, the proline type dipeptide, is extensively used in the medical practise mainly for arterial hypertensivity treatment, congestive heart failure, left ventricular dysfunction and renovascular diseases. It is a prodrug which is after peroral administration rapidly metabolised via enzymatic hydrolysis to the active substance diacid enalaprilat. Enalapril is quickly absorbed from GIT and both enalapril and enalaprilate reach very low concentration levels in blood which are difficult to detect by common UV detection techniques.

Methods For the drug level monitoring highly sensitive and specific method of LC-MS was developed. 3D ion trap was utilised in detection. The strategy of method development was focused on:

1) the choice of structurally similar internal standards for both parent drug and its metabolite,
2) optimisation of the HPLC separation of all four analytes in total run time less than 15 min,
3) selective sample pretreatment by solid phase extraction.

We had optimised the separation under varied chromatographic conditions: temperature, pH and the concentration of the buffer (ammonium formiate), organic solvents (methanol, acetonitril), while elution gradient and flow (0.2 mL/min) of the mobile phase remained constant. Two stationary

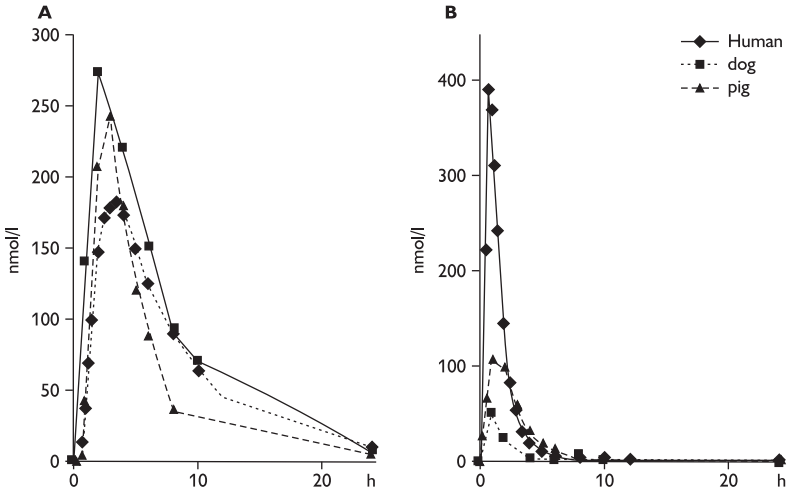

Figure 1 – Comparison of the average pharmacokinetics of enalaprilat (A) and enalapril (B) in human, dog and pig.

reversed-phases, pentafluorophenyl propyl (Discovery® HS F5, Supelco) and octyl (BetaBasic-8, ThermoElectron Corporation), were compared. Separation properties of these phases substantially differed from each other, temperature and the pH dependence characteristics were the most significant.

The matrix effect removal played the key role and critical step of analysis we had focused on while achieving the maximum of the recovery at serum sample preparation by solid phase extraction.

Results and Conclusions Validation parameters of the method complied with the requirements for pharmacokinetic measuremnets. LLOQ of 1 and 0.3 pmol/ml serum for enalaprilat and enalapril respectively were achieved. The method was applied to pharmacokinetic studies in pig, beagle and human Figure 1.

The Positive Influence of Pycnogenol on the Hearts of STZ-induced Diabetic Rats Is Not Dependent on its Antioxidant Potential

Yaghi D., Jankyová S., Priesolová E., Hamáková B., Klimas J., Kyselovič J., Mátyás S.

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Key words: Pycnogenol – Diabetes – Oxidative stress – Antioxidants – Cardiovascular disease

This project was supported by the grant VEGA SR č. 2/5129/25, UK/283/2007 and UK 315/2007.

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Introduction Pycnogenol, one of the strongest antioxidant, is standardized extract from french maritime pine bark (Pinus maritima). In our previous experiment, pycnogenol exhibited positive influence on the impaired contractility of left ventricle in rats with streptozotocin (STZ) induced diabetes. Because sevral authors describe the changes of expression of endothelial nitric oxide synthase, inducible nitric oxide synthase (eNOS and iNOS), tubulin and gp^{91}phox (NOX2) in association with increased oxidative stress in diabetes, in following experiment we observed, whether pycnogenol influences the expression of these proteins in the left ventricle of myocard in the same experimental model of diabetes.

Methods Wistar rats were treated with STZ (25 × 3 mg/kg, i.p., in 24-h intervals) to induced diabetes. Therafter, pycnogenol (50 mg/kg/day, p.o.) was administered 8 weeks in the STZ+PYC group. Conrol rats received vehiculum (CON). We observed significant elevation of plasma blood glucose in diabetic rats during the experiment. The expression of eNOS, iNOS, tubulin and NOX2 in the left ventricle were measured with SDS-PAGE and Western blotting. The expression of eNOS and tubulin were normalized on actin, the expression of iNOS and NOX2 on actinin (CON=100%). Data were expressed as mean ± standard error of the mean.

Results and Conclusions The expression of NOX2 and tubulin in group with experimental diabetes was significantly increased compared to control group (177% ± 17%, $p < 0.05$ vs. CON and 138±7%, $p< 0.05$ vs. CON, Figure 1). In treated group we observed a trend towards decreased expression of NOX2 (156% ± 14%, Figure 1). The expression of tubuline was not changed in the treated group (136% ± 9%, $p < 0.05$ vs. CON, Figure 1). We observed any changes in the expression of iNOS and eNOS in any group (Figure 2).

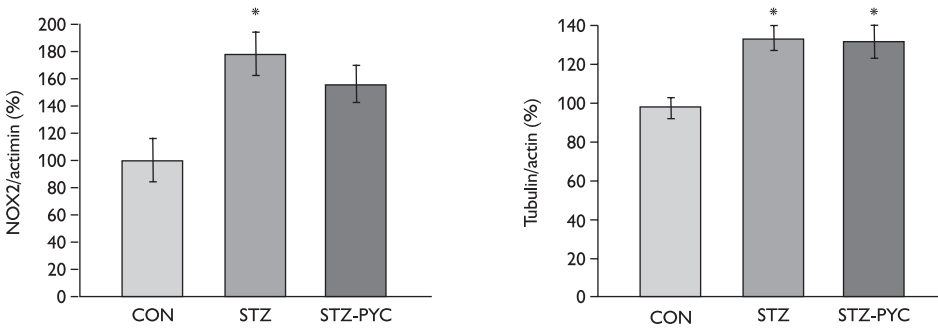

Figure 1 – Expression of NOX2 and tubulin, p< 0.05 vs. NOC.

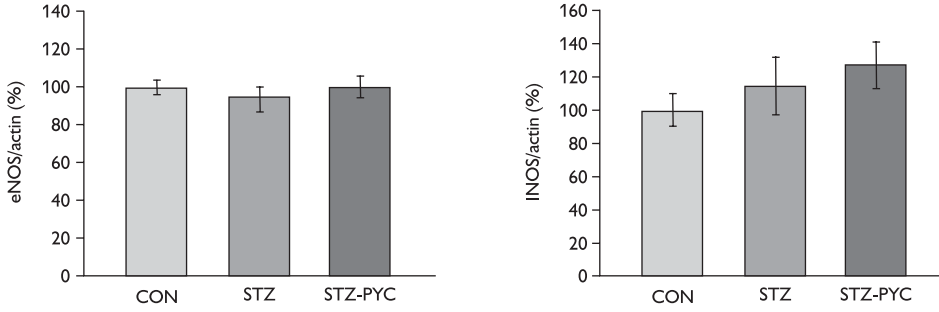

Figure 2 – Expression of eNOS and iNOS.

Pycnogenol did not influence the increased expression of NOX2 and tubulin in experimental model of DM. Therefore we suppose, that the positive effect of pycnogenol on left ventricle functions is independent from its antioxidant activity.

Isoprenaline Cardiotoxic Insult is Aggravated by Rutin

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Key words: Rutin – Flavonoids – Isoprenaline – Catecholamine – Cardiovascular diseases

This work was supported by a Grant Agency of Charles University, No. 39207 C.

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Introduction The synthetic catecholamine isoprenaline (ISO) is often used in experimental cardiology for induction of a pathological state with many similarities to acute myocardial infarction. The mechanisms of ISO cardiotoxicity are not fully understood, but reactive oxygen species (ROS) generated especially by participation of free iron play a role. Therefore, the aim of this study was to test whether rutin with its known iron binding and ROS-scavenging properties may revert ISO-cardiac injury.

Methods 65 Male Wistar: Han rats were randomly divided in 6 groups: controls (saline only), ISO (100 mg/kg s.c.), rutin in a dose of 46 mg/kg i.v. (Ru46) and in ¼of previous dose (Ru11), combination groups premedicated with rutin before application of ISO – RuI46 and RuI11. Haemodynamic parameters were measured after 24 hours. Heart was removed for myocardial element analysis and blood withdrawn for assessment of cardiac troponin T (cTnT).

Results and Conclusions No mortality was observed in the controls and animals who received rutin only. ISO caused 31% mortality. Rutin in a dose of 11.5 mg/kg did not affect this parameter (27%), while fourfold dose increased the mortality to 53%. These results were in concordance with cTnT and myocardial calcium content. Moreover, the larger dose of rutin brought about an

increase in myocardial calcium. There were not statistically significant changes in diastolic or systolic blood pressures in all groups although both doses of rutin lowered total peripheral resistance augmented by ISO. Heart rate increased solely in combination group Rul46.

Conclusively, rutin did not improve myocardial impairment caused by ISO, in addition, larger dose seemed to aggravate it.

Trans-resveratrol Enhances Sex Differences in CYP1A2 Metabolic Activity in Rats

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Key words: *Trans*-resveratrol – CYP1A2 – Sex difference

This work was supported by the by the Czech Science Foundation grant project 525/06/1757.

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Introduction *Trans*-resveratrol (*t*-RES) is a polyphenolic compound present in a variety of plants. *T*-Res elicits many actions in human body which are considered to be protective. Some authors reported *t*-RES influence on the activity of cytochrome P450 (CYP450). It is one of the major enzymatic systems. Many substances including drugs are metabolized by CYP450. Its activity depends on many factors e.g. genotype, age, sex, or xenobiotics. Changes in CYP450 activity can play an important role in drug effectiveness and toxicity, thus knowledge of xenobiotic influence on this enzyme is important for predicting drug interactions.

The aim of our study was to evaluate the influence of sex difference and *t*-RES administration on activity of CYP1A2 in rats.

Methods Male and female Wistar albino rats were used. Animals weighing 200 ± 20 g were housed under controlled conditions with free access to food and water. Animals were divided into 4 groups. Two groups were males and two females. Resveratrol pre-medicated male (RM) and female (RF) animals were administered intraperitoneally by *t*-RES dissolved 30% DMSO at the dose of 5 mg/kg/day for 10 days. Control female (CF) and male (CM) rats were administered by i.p. injections of 30% DMSO in an appropriate volume.

We used model of isolated perfused rat liver for analysis of CYP1A2 activity. The recirculating apparatus was constructed according to the principles of Miller. Marker – phenacetine (PHE) (10.0 mg/l) was added into the perfusion medium. Samples were collected at the 30th, 60th and 120th min of perfusion. Quantitative analysis was performed by HPLC.

Results and Conclusions Our results documented influence of sex on the CYP450 activity. Levels of PAR were significantly increased in CF group ($p \leq 0.05$), which means that females metabolized PHE faster than males (CM).

The activity of CYP1A2 in *t*-RES rats was similar to control animals. *T*-RES didn't influence the activity in animals of same sex, but interestingly enhanced the sexual difference. PAR in perfusate of *t*-RES females was significantly higher than in *t*-RES males. This result is similar to that found in control animals, but statistical significance of this effect was $p \leq 0.001$.

We confirmed our suggestion, that *t*-RES can modulate activity of CYP1A2 in dependence of sex. Our hypothesis was based on the estrogenic effect of *t*-RES. Our opinion is that estrogenic mechanism of CYP450 modulation is the crucial factor of differences in sex dependent CYP450 activity.

Alteration of Liver Regeneration and Cholesterol Homeostatic Mechanisms in Rats after Partial Hepatectomy and Hypercholesterolemic Diet

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Key words: Partial hepatectomy – Cholesterol homeostasis

The financial support by the grants from IGA MZ CR NR/8500-3 and MSM ČR No. 0021620820 is gratefully acknowledged.

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Introduction High dietary cholesterol intake is the common problem in the developed countries, which contributes to increased mortality due to

cardiovascular but also other organ system impairment. The pivotal role in the regulation of the whole-body cholesterol homeostasis plays the liver, where the cholesterol processing is multiplex and includes the synthesis of cholesterol and cholesterol-carrying apoproteins; catabolism of cholesterol to bile acids; receptor-mediated clearance of cholesterol containing lipoproteins; and esterification of cholesterol. Not surprisingly, high cholesterol dietary intake not only induces the alteration of these hepatic processes but also activates profibrogenic signalling in the liver which may lead to progression of hepatic impairment and possibly to worsening of liver regeneration capacity. Therefore, the aim of the present study was to evaluate the impact of long-term dietary cholesterol overload on the cholesterol homeostasis and liver regeneration in rats.

Methods Serum lipid parameters, tissue ^{14}C-cholesterol incorporation, liver DNA synthesis and protein expression of Acyl-CoA:cholesterol acyltransferase-2 (ACAT), LDL receptor, cytochrome CYP7a1, HMG-CoA reductase, SR-B1 receptor, Abca1, Abcg5 and Abcg8 transporters was determined in intact and partially hepatectomized (PH) rats fed with a standard (SLD) or hypercholesterolemic (CHOL) diet.

Results and Conclusions 29-day continual intake of high cholesterol diet in rats before PH produced 135%, 236%, and 250% increase in serum total cholesterol, triglyceride and LDL lipoprotein concentration, respectively. PH provoked decrease in serum total cholesterol, and triglyceride concentration in both SLD and CHOL groups. PH was associated with increase in serum ALT activity more pronounced in CHOL animals. Hepatic DNA synthesis was increased after PH in both dietary groups, nevertheless, PH in CHOL animals yielded lower induction of liver DNA synthesis than in SLD groups. Importantly, hypercholesterolemic diet reduced the activity of orally applied radiolabeled cholesterol in intestine, blood and liver. The ^{14}C-cholesterol hepatic activities tend to increase after PH in both diet groups. Long-term CHOL diet produced down-regulation of LDL receptor and up-regulation of ACAT and SR-B1 protein expression. PH was associated with marked increase in LDL receptor and ACAT and decrease in SR-B1 and Abca1 protein expression in both dietary groups. The expression of Abcg5 and Abcg8 efflux transporters was reduced after PH only in SLD animals. In conclusion, our data showed that liver regeneration after PH is negatively influenced by high cholesterol diet in rats as indicated by changes in serum biochemical parameters and DNA synthesis in the liver. Increased uptake and utilization of cholesterol in the liver after PH is supported by LDL receptor and ACAT up-regulation and down-regulation of Abca1, Abcg5 and Abcg8 protein expression. In addition, decreased cholesterol absorption from GIT and reduction of its liver uptake are suggested as potential compensatory mechanisms during high cholesterol dietary intake.

Nominal Index

Prague Medical REPORT

(Sborník lékařský)

Published by the First Faculty of Medicine, Charles University in Prague,
The Karolinum Press, Ovocný trh 3, 116 36 Praha 1 – Staré Město, Czech Republic.
Vice-Rector-Editor: Prof. Mojmír Horyna, MA., PhD.

Editorial office: Prague Medical Report, U Nemocnice 4, 128 52 Prague 2,
Czech Republic, Phone +420 224 965 670, Phone/Fax +420 224 965 674,
e-mail: medical.report@lf1.cuni.cz
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The Supplement for the 58th Pharmacological Days was prepared
by Dalibor Černý, PharmD.
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Published as quarterly journal. Typesett by MU studio, Radlická 1/19, 150 00 Prague 5.
Printed at Tiskárny Havlíčkův Brod, a. s., Husova 1881, 580 01 Havlíčkův Brod.
Annual subscription (4 issues) EUR 60,–. Single copy EUR 30,–.
Subscription information: Mediaservis s.r.o. For ČR – Zákaznické centrum,
Moravské náměstí 12D, 659 51 Brno, phone for receipt of orders and changes
+420 541 233 232, phone for claims +420 800 800 890, fax +420 541 616 160,
e-mail: zakaznickecentrum@mediaservis.cz. Abroad – Centrum administrace a vývoz tisku,
Sazečská 12, 225 62 Praha 10, phone +420 271 199 250, fax +420 271 199 902,
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ISSN 1214-6994

Reg. No. MK ČR E 796