SEKCE
BIOLOGICKÝCH VĚD

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Středa 22. 4. 2015
(Posluchárna A)

8:15 Slavnostní zahájení konference (Posluchárna A)

8:30 Název přednášky: **Effect of amygdalin activated with β-D-glucosidase on HeLa, MCF-7 and PC-3 cancer cells proliferation**

Autor přednášky: Marie Janatová
Katedra: Katedra farmaceutické botaniky a ekologie
Školitel: prof. RNDr. Luděk Jahodář, CSc.

8:45 Název přednášky: **The effect of epigallocatechin gallate on the induction and repair of the DNA damage induced by hydrogen peroxide in human adenocarcinoma cells A 549**

Autor přednášky: Terézia Kúdelová
Katedra: Katedra biochemických věd, Katedra toxikologie a vojenské farmacie, FVZ
Školitel: doc. Ing. Barbora Szotáková, Ph.D., prof. RNDr. Rudolf Štětina, CSc.

9:00 Název přednášky: **Development of nematodes resistence to albendazole**

Autor přednášky: Tereza Dědková
Katedra: Katedra biochemických věd
Školitel: prof. RNDr. Lenka Skálová, Ph.D.

9:15 Název přednášky: **Selection of reliable reference genes for real-time PCR studies in Haemonchus contortus**

Autor přednášky: Michaela Růžičková
Katedra: Katedra biochemických věd
Školitel: Ing. Petra Matoušková, Ph.D.

9:30 Název přednášky: **Cytomegalovirus infection with HCMV strain and its relationship to the immunosuppression**

Autor přednášky: Aneta Nová
Katedra: Katedra molekulární patologie a biologie, FVZ
Školitel: Mgr. Klára Kubelková, Ph.D.

9:45 Název přednášky: **UHPLC-MS/MS absolute quantification of cytochrome P450 enzymes in C3A, CACO2 modified cell lines and in human liver microsomes**

Autor přednášky: Lenka Rejšková
Katedra: Katedra biochemických věd
Školitel: doc. Ing. Barbora Szotáková, Ph.D.

10:00 Přestávka

10:15 Název přednášky: **Interaction of antiretroviral drugs etravirine and rilpivirine with ABC drug efflux transporters in vitro**

Autor přednášky: Valeria Zelinscaia
Katedra: Katedra farmakologie a toxikologie
Školitel: PharmDr. Martina Čečková, Ph.D.

10:30 Název přednášky: **Influence of TNF-α on hENaC subunits expression**

Autor přednášky: David Martan
Katedra: Katedra biochemických věd
Školitel: prof. Rosa Lemmens-Gruber, PharmDr. Hana Bártíková, Ph.D.

10:45 Název přednášky: **Copper chelating and reducing effects of quercetin metabolites**

Autor přednášky: Jakub Mísař
Katedra: Katedra farmaceutické botaniky a ekologie
Školitel: PharmDr. Jana Karlíčková, Ph.D.

11:00 Název přednášky: **In vitro cultures of medicinal plants – Silybum marianum**

Autor přednášky: Lucie Cinková
Katedra: Katedra farmakognozie
Školitel: doc. PharmDr. Lenka Tůmová, CSc.

11:15 Název přednášky: **Interaction of antiretroviral drug abacavir with drug efflux ATP-binding cassette (ABC) transporters**

Autor přednášky: Ondřej Martinec
Katedra: Katedra farmakologie a toxikologie
Školitel: PharmDr. Lukáš Červený, Ph.D.

11:30 Název přednášky: **Effect of sulforaphane on biotransformation enzymes in rat**

Autor přednášky: Andrea Dymáková
Katedra: Katedra biochemických věd
Školitel: doc. Ing. Barbora Szotáková, Ph.D.

11:45 Přestávka

12:00 Název přednášky: **Characterization of human warfarin reductase**

Autor přednášky: Simona Sokolová
Katedra: Katedra biochemických věd
Školitel: PharmDr. Petra Malátková, Ph.D.

12:15 Název přednášky: **Study of effects of commonly used antiretrovirals on ABCG2-mediated transport of abacavir across cell monolayer**

Autor přednášky: Lenka Ťupová
Katedra: Katedra farmakologie a toxikologie
Školitel: PharmDr. Lukáš Červený, Ph.D.

12:30 Název přednášky: **Effect of sesquiterpenes on biotransformation enzymes in tissue slices**

Autor přednášky: Tomáš Zárybnický
Katedra: Katedra biochemických věd
Školitel: doc. Ing. Barbora Szotáková, Ph.D.

12:45 Název přednášky: **Characterization of LRRK2-mutant iPSC-derived astrocytes**

Autor přednášky: Zuzana Maděryčová
Katedra: Katedra farmakologie a toxikologie
Školitel: PharmDr. Marie Vopršalová, CSc.

13:00 Název přednášky: **Solubilisation, Purification and Reconstitution of Human 17-beta-hydroxysteroid dehydrogenase type 3 (HSD17B3)**

Autor přednášky: Tereza Zahradníková
Katedra: Katedra biochemických věd
Školitel: RNDr. Lucie Zemanová, Ph.D.

13:15 Název přednášky: **Verification of the efficacy of the herbal blend defined PD007 to accelerate ethanol metabolism and decrease its toxic effects on the human organism**

Autor přednášky: Květa Macharová
Katedra: Katedra vojenského vnitřního lékařství a vojenské hygieny
Školitel: Ing. Hana Střítecká, Ph.D.

13:30 Přestávka na oběd

14:15 Název přednášky: **Human membrane-bound enzymes as targets of racin-immobilized affinity carrier**

Autor přednášky: Radka Štýbnarová
Katedra: Katedra biochemických věd
Školitel: RNDr. Lucie Zemanová, Ph.D.

14:30 Název přednášky: **Circadian rhythm sleep disorder**

Autor přednášky: Pavla Koláčková
Katedra: Katedra biologických a lékařských věd
Školitel: doc. RNDr. Vladimír Semecký, CSc.

14:45 Název přednášky: **Generation of DNA constructs for the study of gene regulation via nuclear receptors**

Autor přednášky: Štefan Moravčík
Katedra: Katedra farmakologie a toxikologie
Školitel: prof. Petr Pávek, Ph.D.

15:00 Název přednášky: **Alkaloids of selected Galanthus, Leucojum and Narcissus species and their biological aktivity**

Autor přednášky: Kateřina Breiterová
Katedra: Katedra farmaceutické botaniky a ekologie
Školitel: doc. Ing. Lucie Cahlíková, Ph.D.

15:15 Název přednášky: **Changes of MPV during end-stage renal failure: A link between platelet size, inflammation and main causes of chronic kidney dinase**

Autor přednášky: Barbora Vaňková
Katedra: Katedra biologických a lékařských věd
Školitel: prof. MUDr. Jaroslav Malý, CSc.; PhDr. Zděnka Kudláčková, Ph.D.

15:30 Název přednášky: **Synthesis of tetrazole derivatives with high antimycobacterial activity and their initial in vitro toxicity assessment**

Autor přednášky: Věra Kavková
Katedra: Katedra biochemických věd
Školitel: Mgr. Miloslav Macháček

15:45 Název přednášky: **Pharmacological evaluation of potential Alzheimer´s disease drugs**

Autor přednášky: Lenka Matoušková
Katedra: Katedra farmakologie a toxikologie
Školitel: PharmDr. Marie Vopršalová, CSc.

16:00 Přestávka

16:15 Název přednášky: **The entrance of intracellular pathogen Francisella tularensis into B cells**

Autor přednášky: Barbora Hadámková
Katedra: Katedra biologických a lékařských věd, Katedra molekulární patologie a biologie (FVZ)
Školitel: Mgr. Klára Konečná, Ph.D., plk. doc. RNDr. Zuzana Kročová, Ph.D.

16:30 Název přednášky: **Screening for cytotoxic activity of Amaryllidaceae alkaloids**

Autor přednášky: Zuzana Kavková
Katedra: Katedra farmaceutické botaniky a ekologie
Školitel: doc. Ing. Lucie Cahlíková, Ph.D.

16:45 Název přednášky: **Expression of DHRS8 and DHRS12 enzymes in human tissues**

Autor přednášky: Markéta Svobodová
Katedra: Katedra biochemických věd
Školitel: Mgr. Eva Novotná, Ph.D.

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EFFECT OF AMYGDALIN ACTIVATED WITH Β-D-GLUCOSIDASE ON HELA, MCF-7 AND PC-3 CANCER CELLS PROLIFERATION.

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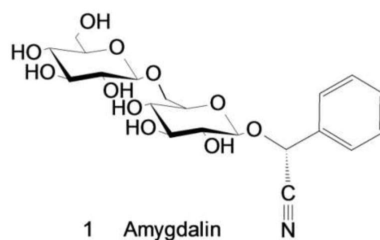
Stone fruits from tribe Amygdaleae of Rosaceae family are known for their antioxidant activity and amount of nutrients and vitamins. Their seeds are connected with content of cyanogenic glycoside amygdalin and its possible effect on inhibition of cancer cells growing.

The anti-proliferative activity brought by stand-alone amygdalin (**1**) and amygdalin activated with β-D-glucosidase from almonds was evaluated in HeLa (cervical), MCF-7 (breast) and PC-3 (prostatic) human cancer cell lines. The MTT viability assay showed that all samples inhibited growth of all three cell lines in dose and time dependent manners. IC_{50} values on the proliferation of the three cell lines for 24 h were more than 15 mg/mL for stand-alone amygdalin and less than 7 mg/mL for amygdalin combined with β-D-glucosidase.

In vitro degradation study of amygdalin with β-D-glucosidase was examined by rp-HPLC to characterize enzymatic hydrolysis rate. Experiments showed that amygdalin could be decomposed to benzaldehyde during the first 1.5 h. Optimum reaction conditions were determined as follows: 37°C, phosphate buffer system (pH 7.4), the ratio of amygdalin/enzyme 1 : 0.12. The results indicate that amygdalin in combination with β-D-glucosidase significantly inhibit *in vitro* growth of the carcinoma cells.



The study was supported by the Charles University, SVV 260 184.

THE EFFECT OF EPIGALLOCATECHIN GALLATE ON THE INDUCTION AND REPAIR OF THE DNA DAMAGE INDUCED BY HYDROGEN PEROXIDE IN HUMAN ADENOCARCINOMA CELLS A549

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During their life, human cells are exposed to oxidative stress. The cell damage caused by reactive oxygen species (ROS) has been recognised as a major cause of cell ageing and the subsequent mutagenesis. The aim of our work was to identify the protective effects of epigallocatechin gallate (EGCG) on DNA in human lung cells A549 under oxidative stress generated by hydrogen peroxide (H_2O_2).

The A549 cells were treated with EGCG at several concentrations for one hour and subsequently exposed to hydrogen peroxide at different concentrations. In this way, the protective effect of EGCG against H_2O_2 -induced damage was studied. Analogically, the DNA repair process was followed with A549 cells first exposed to hydrogen peroxide at several concentrations and subsequently incubated with EGCG at different concentrations for two reparation periods – 15 and 30 minutes. The impaired oxidised bases were detected by enzymes, endonuclease III (Endo III) and formamidopyrimidine-DNA-glycosylase (Fpg). The oxidative damage to DNA was assessed quantitatively using the Comet Assay method.

In the first case, the protective effect of EGCG against hydrogen peroxide induced DNA damage was confirmed. The A549 cells were treated with EGCG at concentrations from 12.5 up to 50 µg/ml, for one hour and then exposed to H_2O_2 at concentrations 0, 10, 25, 100 µM for 5 minutes. EGCG at concentrations, 25 and 50µg/ml has protective effect on DNA damage caused by oxidative stress compared to the control (no EGCG pre-treatment).

In the second case, A549 cells were exposed to H_2O_2 at concentrations of 0, 50, 100, 200 μM for 5 minutes, and afterwards they were treated with EGCG at concentrations of 12.5 and 25 μg/ml during two reparation periods: 15 and 30 minutes. The oxidised bases were detected using enzymes Endo III and Fpg. EGCG was found improving the reparation, however, the mechanism underlying this phenomenon has not been identified so far.

The study was supported by the Charles University, SVV 260 186

DEVELOPMENT OF NEMATODES RESISTANCE TO ALBENDAZOLE

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Nematodoses, including haemonchosis, disease caused by *Haemonchus contortus,* are responsible for substantial losses in livestock farming. *Haemonchus contortus* inhabits the abomasum of small ruminants and causes anemia and gastritis. Currently available anthelmintics used to treat haemonchosis are ineffective in many breeds because the increasing incidence of multiresistant strains of *Haemonchus* worldwide. Therefore, the research of the mechanism of drug resistance of these parasites is very actual and important. The aim of my work was to study whether short-term contact of eggs or adults of *Haemonchus* with anthelmintics can affect the expression of certain enzymes and can lead to development of resistant individuals. The study had two parts.

In the first part, the influence of anhelmintic drug albendazol and its main metabolite albendazol sulfoxide on *Haemonchus* eggs isolated from the feces of infected sheep was tested. In the second part, expression changes of the reference and selected genes in adult males and females of *Haemonchus* from two strains with different sensitivity to anthelmintics were studied. One group of adults of both genders and strains were exposed to culture medium with 10μM albendazole for 12 and 24 hours. ABZ-untreated group (controls) were exposed to culture medium without drugs for 12 and 24 hours. The genes for UGTs (UDP-glucosyl trasferases) were monitored, because in a previous study, higher UGTs activity and increased ability to deactivate albendazole via conjugation with glucose was found in the drug-resistant strain than in sensitive strain.

In my work, I tested the expression of UGT7, 12 and 13, but I didn´t find statistically significant differences in their expression between strains. The contact of *Haemonchus* with albendazole also did not affect expression of these enzymes. For that reason, tested enzymes probably don´t contribute to increased metabolism of albendazol in a resistant strain. Due to a large amount of UGTs (more than 40) in *Haemonchus*, it´s possible that deactivation of albendazole is catalyzed by other UGTs. Study of UGTs is a part of an ongoing project which deals with mechanisms of helminths resistance and which promises other more interesting results.

The study was supported by SVV 260186.

RELIABLE REFERENCE GENE SELECTION FOR QUANTITATIVE REAL TIME PCR IN *HAEMONCHUS CONTORTUS*

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Parasite anthelmintic resistance is a great problem of these days. Prophylaxis, treatment and consequences of parasitic infections represents an important economic burden on livestock production worldwide. Mechanisms of drug resistance are still not fully understood. Molecular biology methods, *e.g.* gene expression studies, could contribute to understanding of these mechanisms and thus help in resistance management. Use of suitable reference genes is essential for an accurate normalisation of gene expression levels.

Haemonchus contortus is a parasitic nematode of small ruminants, whose multi-resistance to anthelmintics means global problem. The genome and transcriptome have been published recently, allowing extensive gene expression research to be conducted. Suitable reference genes for different strains of *H. contortus* have not been validated yet.

The aim of this work was to identify and validate reliable reference genes for gene expression studies in adult *H. contortus*. 11 candidate genes were chosen for further assessment of their expression stability in males and females of two genetically divergent *H. contortus* strains: drug-susceptible (ISE) and multi-drug-resistant (WR). The candidate genes were selected based on their common use as endogenous controls, supplemented by genes identified bioinformatically based on stable expression in RNA-seq data.

Total RNA was extracted from ten adult *H. contortus* males or females and reverse transcribed to cDNA. An identical reaction without reverse transcriptase was carried out simultaneously. The resulting cDNA was diluted 1 to 50 and used for quantitative real-time

PCR assay (qPCR). iQ5 Real Time PCR Detection System (Bio-Rad, USA) with SYBR green I detection was used for qPCR analyses. Specificity and efficiency of designed primer sets were checked using standard dilutions, efficiency for all primers was between 91-104%.

Expression stability was evaluated by computer programs BestKeeper, geNorm, NormFinder and the comparative ΔΔCt method. Different calculation methods caused slightly different ranking order of genes obtained from each program. However, all methods found ama, farb, gapdh, ncbp and sodc to be the five most stable genes.

By this study we demonstrated, that the combination of commonly used gapdh gene and at least one of the other best ranked genes would be appropriate for gene expression studies in *H. contortus* adults.

The study was supported by the Charles University in Prague (Research project SVV 260 186).

CYTOMEGALOVIRUS INFECTION WITH HCMV STRAIN AND ITS RELATIONSHIP TO THE IMMUNOSUPPRESSION

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The main goal of our study is a contribution to the study of *in vitro* interaction of human cytomegalovirus, belonging to the family *Herpesviridae* with selected immunosuppressed host cells.

During the academic year 2014/2015, we were focused on the infection of human lung fibroblasts MRC-5 with human cytomegalovirus strain VR-1590. During the study, basic laboratory techniques have been used. Among these methods, we were employed to work with cell cultures and viral isolates where the concentration of cytomegalovirus has been quantified using plaque-based assay. Moreover, ionizing radiation was applied to ensure the condition of immunosuppressed host. Subsequently, selected signalling pathways of host cells have been examined in relation to the radiation and/or infection using PathScan antibody technology.

In this study, the cytomegalovirus infection model using immunosuppressed host cells has been introduced and quite well optimized. We have also investigated the target signaling pathways of host cells using specific antibody-determined technology. The relationship between immunosuppressed host cells and viral infection has been studied on the basis of changes of selected transduction pathways signals of these cells. PathScan technology will be further optimized in the context of the study of other selected signaling pathway signals in the future.

This study was supported by Long-term Organization Development Plan 1011 from the Ministry of Defense, Czech Republic.

UHPLC-MS/MS ABSOLUTE QUANTIFICATION OF CYTOCHROME P450 ENZYMES IN C3A, CACO2 MODIFIED CELL LINES AND IN HUMAN LIVER MICROSOMES

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Cytochrome P450 (CYP) enzymes play a crucial role in drug metabolism. They can be responsible for the failure of treatment, adverse and toxic effects or drug-drug interactions. Knowledge of expression levels and their susceptibility to be either induced or inhibited would be the basic tool for personalized therapy. Therefore, *in vitro* and *in vivo* experiments of CYP mediated metabolism is an essential part of the drug development and clinical research.

In vitro studies can be done with primary cells or cell lines. Cell lines are phenotypic stable and immortal but their CYPs levels are low. From this point of view, modified C3A and CACO2 cell lines with constitutive androstane receptor (CAR) and pregnane X receptor (PXR) might be used for these experiments. CYP enzymes should be expressed continuously in these modified cell lines.

With regard to pharmacokinetic and pharmacological importance, the expression levels of metabolizing enzymes CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2E1, CYP3A5 and CYP3A4 were studied in my diploma thesis.

Absolute quantifications of CYP enzymes were carried out by UHPLC in line coupled with tandem mass spectrometry working in scheduled MRM mode. Data assessment was conducted by Skyline 2.6 software.

CYP enzymes were not detected in CACO2 and C3A modified cell lines. However, these enzymes were found in human liver microsomes. Average values were ranging from 0.6

pmol/mg to 21.5 pmol/mg of microsomal protein. The lowest detected amounts of CYP protein were 0.006 – 0.210 pmol/mg of microsomal protein in a hundred times diluted human liver sample. These findings point out that CYPs protein levels in modified C3A and CACO2 cell lines were apparently below the limit of detection.

Results show that up-regulation of CYP enzymes in modified cell lines CACO2 and C3A does not reach CYPs levels in human liver microsomes. Further studies have to be conducted in order to optimize cultivation conditions, presence of co-regulators and ligands to get modified cell lines with measurable CYP levels.

The study was supported by the Charles University, SVV 260 186.

INTERACTION OF ANTIRETROVIRAL DRUGS ETRAVIRINE AND RILPIVIRINE WITH ABC DRUG EFFLUX TRANSPORTERS IN VITRO

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Etravirine and rilpivirine, are relatively new antiretroviral drugs that belong to the second-generation non-nucleoside reverse transcriptase inhibitors used in combination therapy (cART) of HIV positive patients. ABC (ATP-binding cassette) transporters are extensively expressed in normal tissues (e.g. liver, kidney, intestine, blood-brain barrier, placenta), where they are able to affect pharmacokinetic behavior of various drugs. ABCB1 (P-glycoprotein), ABCG2 (BCRP) and ABCC2 (MRP2) represent the most common drug transporters, on which drug-drug interactions (DDI) can occur. Nevertheless, the current knowledge on interactions of etravirine and rilpivirine with the transporters and their potential to create transporter-mediated DDI is only limited so far. In this study we therefore aimed to investigate inhibitory potency of etravirine and rilpivirine towards ABCB1, ABCG2 and ABCC2 drug efflux transporters employing *in vitro* accumulation/efflux studies with relevant fluorescent substrates.

The accumulation assays with Hoechst 33342 and Rhodamine 123 on MCDKII-ABCB1 cells have demonstrated that rilpivirine is a potent ABCB1 inhibitor able to bind H- as well as R-site of the transporter, while etravirine does not inhibit ABCB1 at all. In MDCKII-ABCG2 cells, both antiretrovirals revealed significant inhibitory potency to ABCG2. Nevertheless, using the efflux experiments with calcein-AM in MDCKII-ABCC2 cells neither etravirine nor rilpivirine caused inhibition of MRP2.

Since our data clearly showed that rilpivirine and etravirine are ABCB1 and/or ABCG2 inhibitors, we have additionally employed transport assays to evaluate possible DDI of these drugs with tenofovir disoproxil fumarate (TDF), another antiretroviral drug used in cART and being confirmed as ABCB1 and ABCG2 substrate. Our transport experiments on MDCKII-ABCB1 and MDCKII-ABCG2 cell monolayers demonstrate that both antiretrovirals significantly affect TDF permeability across the cellular monolayer due to inhibition of the ABC drug efflux transporters. These results suggest that etravirine and rilpivirine possess a great potential for drug efflux transporters-mediated DDI, which could have an impact on antiretroviral dosage scheme during cART in clinical practice.

The study was supported by SVV/2015/260-185

INFLUENCE OF TNF-A ON HENAC SUBUNITS EXPRESSION

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The human epithelial sodium channel (hENaC) or the amiloride-sensitive channel, is a type of ion channel which has the ability to control salt and water homeostasis. Therefore it is one of the main driving forces for the reabsorption of water through the alveolar epithelium. A dysfunction of this channel, respectively of this control mechanism, leads to a very severe disease – pulmonary edema and several other pathological conditions.

Previous studies tested a drug named AP301. AP301 is a cyclic protein comprising the human tumour necrosis factor-like domain sequence. This drug was recently developed as a potential treatment of pulmonary edema. The principle is that it activates hENaC by increasing the open probability. It was also shown that AP301 transiently increases the expression of hENaC subunits in mammalian cells.

In this study we used the western blot method to test the influence of tumour necrosis factor α (TNF-α) on hENaC subunits expression and we compared these results with the results from the studies with AP301.

We found that TNF-α transiently significantly increased the expression of δ subunit and it had a potential to increase the expression of α subunit. On the other hand, the expression of β- and γ-hENaC was not significantly increased.

Taken together, these results are analogical to those which were found in the studies with AP301.

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COPPER CHELATING AND REDUCING EFFECTS OF QUERCETIN METABOLITES

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Copper is an essential microelement in particular due to its ability to easily convert between both redox forms: oxidized (Cu^{2+}) and reduced (Cu^{+}).[1] Flavonoids are common components of the human diet and they may positively influence human health.[2] They are converted into smaller phenolic acids during digestion by bacteria in the colon. Although properties of flavonoids have been well studied, the same is not true for their metabolites - phenolic acids.[3]

In this *in vitro* study, 10 phenolic acids, which are known metabolites of commonly tested flavonoid quercetin, were analyzed for their copper chelating activity and copper reducing activity at 4 pathophysiologically relevant pHs. Simple spectrophotometric methods based on hematoxylin and bathocuproinedisulfonic acid disodium salt were used for the assessment chelation and reduction of copper ions.[4]

As expected, metabolites possessing a dihydroxygroup in an *ortho* position were able to chelate cupric ions, however their chelation activity disappeared when challenged with a more powerful copper chelator. The degree of cupric reduction differed among tested compounds. All *o*-dihydroxycompounds were the most active and achieved 100% of cupric ion reduction in low compound to copper ratio.

In conclusion, based on this study, it appears that metabolites of quercetin can influence the kinetics of copper in human.

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IN VITRO CULTURES OF MEDICINAL PLANTS XVII.

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Milk thistle, *Silybum marianum* L. Gaertn., is a source of flavonoid taxifolin and flavonolignans – silymarin complex (silybin, silydianin, silycristin and isosilybin). Due to the the main active component of silymarin - silybin, milk thistle is used as hepatoprotectivum and antioxidant, in skin-health, and in the therapy of some kinds of cancer. New therapeutic potentials of *Silybum marianum* are still discovered.

Milk thistle is usually obtained by field cultivation. Alternative way for getting the active components, is the use of *in vitro* cultures. But the production of secondary metabolites by the *in vitro* cultures is low in comparison with plant. One of the possibilites how to increase this produciton is the method of elicitation.

In this study, ethephon as the elicitor, in the concentrations of 500 µmol/l, 400 µmol/l, 200 µmol/l, 100 µmol/l and 50 µmol/l was used with the aim to increase secondary metabolite production in suspension and callus cultures. The effect of ethephon was compared to its inhibitor (120µM $AgNO_3$). The levels of flavonolignans and taxifolin were measured by the method of HPLC. The samples were taken 24, 48, 72, 96 and 168 hours after the ethephon application and inhibitor treatment. The nutrient medium of suspension culture was also tested for the possibity of secondary metabolites releasing into medium.

The highest content of flavonoid taxifolin was found in the suspension culture medium after 48 h treatment with ethephon in conc. of 400 µmol/l. The level of taxifolin was increased by 197-fold to 1,97 mg/100 ml, compared to control sample.

The statistically significant production of taxifolin in the callus culture was reached after 96 hours of treatment with ethephon in conc. of 50 µmol/l. (0,11 mg/g DW).

The statistically significant production of silybin A was reached in the nutrient medium 72 h after application of 400µM ethephon (0,51 mg/100 ml).

The statistically significant positive effect of $AgNO_3$ as inhibitor was found in the case of taxifolin in the medium, 168 hours after application of 400µM ethephon. Inhibitor increased taxifolin content by 58-fold to 0,58 mg/100 ml.

The statistically significant negative effect of inhibitor $AgNO_3$ was on silybin A content in medium, 168 hours after application of 400µM ethephon. Inhibitor completely decreased the effect of ethephon.

The study was supported by the Charles University, SVV 260 186.

INTERACTION OF ANTIRETROVIRAL DRUG ABACAVIR WITH DRUG EFFLUX ATP-BINDING CASSETTE (ABC) TRANSPORTERS

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Abacavir is a nucleoside reverse transcriptase inhibitor (NRTI) that is frequently used in combination antiretroviral therapy of HIV infection. Pharmacokinetics of many antiretroviral drugs is often affected by the activity of ATP-binding cassette (ABC) transporters. Drug-drug interactions on ABC transporters should be, therefore, always borne in mind as they may complicate therapy. To guarantee effective and safe abacavir-based therapy it is inevitable to have complex knowledge on abacavir interactions with ABC transporters. The aim of our work was to study interaction of abacavir with human drug efflux ABC transporters ABCB1 (P-glycoprotein), ABCG2 (breast cancer resistance protein), ABCC2 and ABCC5 (multidrug resistance-associated protein 2 and 5) using *in vitro* method of MDCKII cell monolayer in setup of bi-directional study and concentration equilibrium. Abacavir was tested at a low non-saturating concentration of 198 nM. Using both experimental setups we observed abacavir active transport across MDCKII-ABCB1 and MDCKII-ABCG2 reaching significantly higher transport ratio after six hours > 2 in bidirectional study and > 2 in concentration equilibrium studies when compared with transport across the parent MDCKII cells. Application of higher concentrations of abacavir (50 µM and/or 100 µM) caused partial saturation of the ABCB1- but not ABCG2-mediated transport. Additionally, it was demonstrated that dual ABCB1/ABCG2 inhibitor GF120918 completely abolished transcellular transport across both MDCKII-ABCB1 and MDCKII-ABCG2 monolayers. We can therefore conclude that abacavir is a substrate of ABCB1 and ABCG2, but not ABCC2 and ABCC5 and it can be hypothesized that ABCB1 and ABCG2 may affect its pharmacokinetics at the level of absorption, distribution and elimination. Additional studies are required to confirm relevancy of abacavir drug-drug interactions on these transporters *in vivo*.

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EFFECT OF SULFORAPHANE ON BIOTRANSFORMATION ENZYMES IN RAT

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Sulforaphane (SF) is a diet-based isothiocyanate, which is occurring in *Brassicaceae* (cruciferous vegetables) for example in broccoli or cabbage. In vegetables it is in the form of glucoside – glucoraphanin. By the following cutting or chewing, it is hydrolysed into the corresponding isothiocyanate SF either by the plant thioglucosidase myrosinase or by bacterial thioglucosidases in the colon. Because of its lipophilicity and molecular size, SF is likely to passively diffuse into the enterocytes. Myrosinase is inactivated by higher temperature. So when we want SF to be absorbed from GIT, the best way to do so is eating raw vegetables or making juice from it. SF has several beneficial effects on human health, e.g. anticancer, antioxidant or neuroprotective effects.

The aim of our study was to evaluate the effect of SF on the activity of selected biotransformation enzymes. The effect of SF was studied on rat liver subcellular fractions and primary culture of rat hepatocytes. Hepatocytes were incubated with SF, with β-naphthoflavone (β-NF) and with SF and β-NF together, all for 24 hours. The activities of conjugating enzymes, reduction enzymes and cytochromes P450 were studied. In subcellular fractions was studied only an inhibitory effect of SF, but in hepatocytes the induction of abovementioned enzymes was studied as well.

Our results show, that SF in concentrations 10μM, 20μM, 50μM and 100μM weakly inhibits glutathione S-transferase (GST) in cytosol. But in contrast, in hepatocytes was observed induction of GST by 10μM SF itself or in combination with β-NF. Sulfotransferase (SULT) and UDP-glucuronosyltransferase (UGT) were not detected in subcellular fractions**.** No effect of SF on UGT activity was proven in hepatocytes too.

In hepatocytes, SF induces aldo-keto reductase (AKR1C) alone or with β-NF. The same results were obtained with carbonyl reductase (CBR) and on top of that, there is a great synergism of SF and β-NF. They induce CBR together more than alone SF. We proved that SF induces NAD(P)H:quinone oxidoreductase 1 (NQO1) in rat hepatocytes and the synergism

of SF and β-NF combination was also observed. In rat liver cytosol SF slightly inhibits NQO1.

The effect of SF on cytochromes P450 (CYPs) was studied as well. In subcellular fractions no effect of SF on CYP1A1 and CYP1A2 activities was proven. In hepatocytes we confirmed the inhibitory effect of SF on CYP1A1, but only when CYP1A1 was induced by β-NF at first. The activity of CYP3A was not found in hepatocytes, in subcellular fractions SF inhibits CYP3A.

The obtained results show that SF affects several biotransformation enzymes in rat. Therefore, sulforaphane appears to be a promising compound with induction effect on detoxifying enzymes.

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CHARACTERIZATION OF HUMAN WARFARIN REDUCTASE

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Warfarin is widely used anticoagulant drug. Considering the narrow therapeutic window of warfarin, it is important to fully understand its metabolism in human body. Oxidative, reductive and conjugation reactions are involved in warfarin metabolism. However, the reductive metabolism of warfarin has not been studied in details until now.

The reduced metabolite of warfarin, i.e. warfarin-alcohol, is produced by the conversion of the carbonyl group of the side chain. It is known that human liver cytosolic and microsomal fractions exhibit warfarin reductase activity but the specific enzymes catalysing the reduction of warfarin are not known yet.

The aim of this study was to identify the enzyme(s) participating in reduction of warfarin and to describe enzyme kinetics. Human liver cytosolic and microsomal fractions and recombinant enzymes AKR1A1, AKR1B1, AKR1B10, AKR1C1, AKR1C2, AKR1C3, AKR1C4, CBR1 and CBR3 were incubated with warfarin at various concentrations. The produced warfarin-alcohol was quantified by UHPLC and the specific activities of enzymes and subcellular fractions were determined.

The warfarin reductase activity was confirmed in cytosolic and microsomal fractions. The reduction of warfarin was higher in the liver cytosol than liver microsomes. From the enzymes tested, AKR1C3 and CBR1 were found as the main enzymes participated in the production of warfarin-alcohol. Other enzymes showed only low or no activity.

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STUDY OF EFFECTS OF COMMONLY USED ANTIRETROVIRALS ON ABCG2-MEDIATED TRANSPORT OF ABACAVIR ACROSS CELL MONOLAYER

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Nucleoside reverse transcriptase inhibitor (NRTI) abacavir is a drug commonly used in combination antiretroviral therapy of HIV infection. It has been previously observed in our laboratory that drug efflux ATP-binding cassette (ABC) transporter, breast cancer resistance protein (ABCG2), recognizes abacavir as a substrate. ABCG2 thus can be an important factor affecting its absorption, distribution, and elimination. As many antiretrovirals are substrates or inhibitors of ABCG2 the aim of our study was to investigate their drug-drug interactions with abacavir on the ABCG2 transporter. For this purpose we used well established *in vitro* model of monolayer formed by MDCKII cells stably expressing ABCG2 in setup of concentration equilibrium. First we analyzed effect of Ko134 (2 µM), model inhibitor of ABCG2, that completely abolished ABCG2-mediated active transport of abacavir across MDCKII monolayer and abacavir was demonstrated to have saturation transcellular kinetics. Subsequently, we tested several concentrations (ranging from 0.1 µM to 100 µM) of eight antiretrovirals (zidovudine, didanosine, lopinavir, atazanavir, ritonavir, stavudine, nevirapine, and rilpivirine) originating from three distinct drug groups (NRTI, non-NRTI, and protease inhibitors). We observed significant inhibition in all cases tested. Protease inhibitors lopinavir (20 µM), ritonavir and atazanavir (both at concentration of 100 µM) and non-NRTI rilpivirine (0.1 µM - 20 µM) showed the highest potency to inhibit ABCG2-mediated transport reaching abacavir was translocated across the MDCKII-ABCG2 monolayer by passive mechanism only. It can be concluded that co-administration of the tested antiretrovirals with abacavir might change pharmacokinetics of abacavir. Our data thus broaden knowledge on abacavir drug-drug interaction, however, it will need to be verified *in vivo*.

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EFFECT OF SESQUITERPENES ON BIOTRANSFORMATION ENZYMES IN TISSUE SLICES

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High-precision tissue slicers (e.g. Krumdieck tissue slicer[1]) allow the rapid production of equally sized tissue slices of less than 250 µm thickness. Tissue slices are viable explants of tissue, cultivated *ex vivo*, with a reproducible and defined thickness and they serve as a multipurpose *in vitro* model. It contains all cell types of the tissue in their natural environment. The thin slices realistically and reliably represent the *in vivo* situation and have been used to study the metabolism, transport and biotransformation of xenobiotics, as well as for toxicological studies and others.

The essential factors, for the viability and function of cells inside the slices, are incubation conditions and slice thickness. These limit sufficient oxygen supply to the inner cell layers and exchange of nutrients and metabolites.

The aim of the study was to evaluate influence of several sesquiterpenes, secondary metabolites mainly produced by higher plants, as possible modifiers of biotransformation enzymes. Sesquiterpenes α-humulene, β-caryophyllene and β-caryophyllene oxide were chosen for this purpose. Above mentioned sesquiterpenes showed a potential as inducers of the detoxifying enzyme glutathione S-transferase in forestomach, liver and small bowel mucosa of A/J mice[2].

In our project we used rat liver slices that were 8 mm in diameter and 200 µm thin. They were cut on the Krumdieck tissue slicer in ice-cold and oxygenated Krebs-Heseleit buffer set up to pH 7.4. After cutting, the tissue slices were incubated for 3, 6 and 24 hours in medium containing sesquiterpene and in carbogen atmosphere (95% O_2/5% CO_2). After, the tissue was

homogenized. The results were compared to control homogenates from slices incubated in clear medium. Advantage of the method lies in producing multiple tissue samples that can be incubated with variable compounds in different concentrations and reducing the number of animals necessary for experiment.

So far, we managed to optimize the method and raise the viability of the tissue slices, especially the viability after 24 hours. The first sesquiterpene studied was α-humulene. However, the results did not show any induction of glutathione S-transferase in comparison to control. We also tried to assess the effect of α-humulene on sulfotransferase and quinone oxidoreductase 1, but the measured activities were at detection limit. Further studies have to be conducted in order to evaluate effect of β-caryophyllene and β-caryophyllene oxide on biotransformation enzymes.

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CHARACTERIZATION OF *LRRK2*-MUTANT iPSC-DERIVED ASTROCYTES

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Somatic cells derived from induced pluripotent stem cells (iPSC) are becoming a model tool of future that allows to study *in vivo* processes under *in vitro* conditions. The protocol for generation of iPSC-derived astrocytes was published only recently. Thanks to this fact, it is possible to study a complicated and not fully understood role of astrocytes under physiological and pathological conditions.

In our pilot study, iPSC-derived astrocytes in the primary research of Parkinson's disease (PD) were used for the first time ever. We focused on comparison of the gene expression profiles of iPSC-derived astrocytes obtained from a healthy individual and a patient with genetically conditioned PD. The G2019S mutation of the *leucine rich-repeat kinase 2* (*LRRK2*) gene was purposely studied. Presence of the *LRRK2* mutation also occurs in some patients with sporadic form of PD and therefore studying this mutation seems to be also beneficial for understanding mechanisms of PD in general.

Quantitative reverse transcription polymerase chain reaction (RT-qPCR), immunocytochemistry (ICC) and western blotting (WB) were employed for an objective analysis of both studied astrocyte lines. These methods analyzed morphological features of iPSC-derived astrocytes, astrocyte-specific markers, disease-associated markers, neuroprotective and pro-inflammatory markers, sensors of organelle functions as well as some enzymes.

Results of our work imply significant changes in astrocyte morphology and gene expression profiles that might be critical in pathology of PD.

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SOLUBILISATION, PURIFICATION AND RECONSTITUTION OF HUMAN 17-BETA-HYDROXYSTEROID DEHYDROGENASE TYPE 3 (HSD17B3)

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Short-chain dehydrogenases/reductases (SDR) superfamily is a large group of NADP(H)/NAD(H)-dependent oxidoreductases. Human SDR enzymes are classified into 47 families, including cytosolic and membrane-bound ones. The objective of this study is a human membrane-bound enzyme 17-beta-hydroxysteroid dehydrogenase type 3 (HSD17B3), which participates in the biosynthesis of steroidal hormones and mainly catalysis the conversion of androstenedione to testosterone. The main goals of this study are to find a suitable detergent for successful solubilisation, purify the enzyme and prepare a reconstitution system for studying of the pure HSD17B3 behavior in the membrane.

Microsomes containing overexpressed HSD17B3 were isolated from Sf9 insect cells (*Spodoptera frugiperda)*. The first step is the solubilisation process, which involves detergent screening. Six detergents were tested, each in final concentration of 0.1%, 0.5% and 1.0% (w/v): ASB 14-4, C12E8, DDM, CHAPS, Igepal CA-630, Triton X-100. The detergent ASB 14-4 in concentration 0.5% (w/v) has been indentified to be the best one for the HSD17B3 solubilisation.

The next step is enzyme purification, using the His10-tag located on C-terminus of the HSD17B3 and Ni-metal affinity chromatography (Ni-IMAC). This method enabled to obtain the pure protein in the concentration 248.92 µg/ml and specific activity 5.16 nmol/mg/60 min based on the reduction of androstendione to testosterone.

The last step was successful incorporation of the *HSD17B3* into custom prepared liposomes, whose phospholipid composition was based on the membrane of the human liver endoplasmic reticulum.

The study was supported by the project SVV 260 186.

VERIFICATION OF THE EFFICIENCY OF THE HERBAL BLEND DEFINED PD007 IN ACCELERATION ETHANOL METABOLISM AND DECREASING ITS TOXIC EFFECTS ON HUMAN ORGANISM

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This pilot evaluation assesses effects of using specific herbal blend, expecting to relieve the symptoms of organism intoxication with ethanol and accelerate ethanol metabolism.[1][4][5]

The aim of this study is to evaluate effects of the administration of standardized herbal blend tested on selected subjective and objective parameters of alcohol intoxication and monitoring the time course of alcohol levels changes in blood with or without tested herbal blend.

The herbal blend consists of Hovenia Dulcis, Pueraria Lobata, Panax Quinquefolius.

There are studies confirming the efficiency of individual plants in therapy of alcohol intoxication. Testing was performed on mice. [1][2][4][5][7]

Scientists found that the main substance, dihydromyricetin, blocks receptors in the brain responsible for transmission of aminobutyric acids (GABA). Ethanol isn't bound on neurons and remains in the bloodstream, where it can be transferred to livers for further degradation.[1] As referred in the study: Mice remained sober in spite of excessive level of alcohol concentration in blood. [1][2][4][5] DHM accelerates liver ability to degrade both alcohol, and the toxic metabolite acetaldehyde.[1][2][3][4]

Substances contained in Kudzu root extract inhibits or modify the effects of alcohol dehydrogenase. It was also confirmed that isoflavonoids, found in root of Kudzu, increase

concentration of serotonin and dopamine in the CNS, which leads to a gradual reduction of necessity to alcohol consumption in any form.[7][8][9][10]

The tested subjects use the herbal blend, which is in the form of a dry powder. Tested subject drinks a defined dose of ethanol with given alcohol concentration. The amount of the dose is calculated referring to body weight using specific mathematical formula. The aim is to achieve peak blood levels of alcohol in the range of 0.5 -1 per mille. The level measuring is carried out by breathalyser. The part of monitoring the effect of alcohol on organism is performing the response test. The test shows changes in ability to focus on performance and in changes of fine motor coordination. Alcohol breath test is carried out every thirty minutes until the alcohol level is below 0.1 per mille. In the first test scheme the subjects are administered alcohol only. The values obtained will serve as a control measurement in further examinations of ethanol degradation. In the second test scheme the subject first uses the tested herbal blend and after thirty minutes drinks a specified dose of alcohol (the same dose as in scheme one). In the third test scheme monitored subject drinks alcohol (the same dose as in tests one and two) and thirty minutes from administering of alcohol the tested herbal blend is applied. According to the results of the tests number 2 and 3 the most successful scheme is selected and it will be performed with doubled dose of the herbal blend.

Data analysis evaluated referring to the duration of decreasing alcohol level below 0.1 per mille in relation to sex, body constitution and results of the response test.

The study would be important for diagnostic or therapeutic methods for medical science. [6]

The results can’t be assessed at this very moment, but it is believed that one dose of herbal blend should be taken before alcohol consumption and another dose after finishing of the consumption even before going to bed to achieve maximal efficiency of the blend. If the tests will confirm the assumptions about the positive effect of the blend on reducing the symptoms of intoxication and acceleration of ethanol metabolism stated in literature than the results will serve as initiation of a broader study. The main objective of upcoming study would be obtaining objective data for possible use of the bland as a complementary therapy. [1] [6][8][10]

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HUMAN MEMBRANE-BOUND ENZYMES AS TARGETS OF ORACIN-IMMOBILIZED AFFINITY CARRIER

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Carbonyl reducing enzymes play important role in the metabolism of various eobiotic (e.g. steroids, prostaglandins) and xenobiotic (e.g. doxorubicin, daunorubicin, oracin, NNK, haloperidol) compounds. Due to their substrate specificity, they also play a role in development of some diseases like hormone-dependent cancers and metabolic syndrome. While cytosolic carbonyl reducing enzymes are well characterised the knowledge about membrane-bound types is quite poor because their study is demanding.

Actually, until today there are only three described microsomal carbonyl reducing enzymes participating in the metabolism of xenobiotic compounds (11β-HSD1, DHRS7 and DHRS3)[1,2] of which only the 11β-HSD1 is well characterized. However, based on the research of anticancer drug oracin reduction stereospecificity, there were predicted other microsomal carbonyl reducing enzymes involved in its metabolism and inactivation[3].

In order to isolate these "unknown" enzymes the in-house developed affinity carrier with immobilized ligand oracin[4] was introduced into the purification protocol of human liver membrane-bound enzymes[3]. Proteins having affinity towards oracin were successfully captured by our affinity carrier and subsequently gently eluted by 100 mM glycine buffer, pH 10.5. Using mass spectrometry enzyme 17β-HSD6 was identified. Despite its metabolism of eobiotics (e.g.retinol, testosterone and estradiol) was already described there are still no published information about its role in the metabolism of xenobiotics. Thus its isolation and

identification as a potential target of drug oracin could significantly extend our knowledge about its role in biotransformation.

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CIRCADIAN RHYTHM SLEEP DISORDER

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Current lifestyle with more stress factors helps to impair physiological programming of circadian rhythm, because of that the number of patients with sleep disorders is growing.

Study was conducted at the Centrum of disorders of sleep and biorhythm in University hospital in Hradec Králové, Czech Republic in co-operation with doc. MUDr. Petr Smolík. A total of 51 patients were enrolled in the study, each of them was controlled minimally once every 3 months. The two main groups are insomnia and circadian rhythm sleep disorder patients.

Method of study was the personal contact with patients, together filling in questionnaires and follow up work with medical history and consultation with psychiatrist from sleep laboratory.

Except standard sleep distribution, we distinguish patients with advanced sleep phase syndrome – ASPS (larks) or delayed sleep phase syndrome - DSPS (owls). Patients with ASPS aren't limited in social and economical life as much as patients with DSPS. Main problems of DSPS are demonstrated on two, to point out complexity of therapy in patients with DSPS.

The cases are focused on physiological programming and individualization of therapy, especially using right dosage of drug at the right time. Therapy is also based on complex change of patient’s lifestyle, which is beneficial for enhancing patient’s compliance and adherence not only to pharmacological part, but also to psychological part of therapy. Incorrectly administered dosage of drugs, usually associated with drug abuse, might not help patient, in one given case study it made patient´s condition worse.

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GENERATION OF DNA CONSTRUCTS FOR THE STUDY OF GENE REGULATION VIA NUCLEAR RECEPTORS

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Nuclear receptors PXR, HNF4α and CAR are transcription factors that control expression of major xenobiotic-metabolizing enzymes and transporters.

The gene reporter assay is widely used method in the study of gene regulation through nuclear receptors. The methods is based on a gene reporter DNA construct with target gene promoter sequence upstream of a firefly luciferase or green fluorescence protein (GFP).

In my project I introduced GENEART^{TM}Site-Directed Mutagenesis and DNA plasmid ligation techniques to generate the following DNA constructs:

(i) the organic cation transporter-1 (OCT1) luciferase reporter constructs with mutated HNF4α response elements for the study of OCT1 regulation;

(ii) gene reporter (both luciferase and GFP) constructs for cytochrome P450 CYP3A4 gene, a target gene regulated by PXR and CAR;

(iii) generation of Constitutive androstane receptor (CAR) chimera expression construct with extra alanine residue in CAR ligand binding domain and enhancer green fluorescence protein (EGFP) for the study of CAR-mediated regulation of CYP3A4 gene and CAR activation

I generated three OCT1 luciferase gene reporter constructs with mutated HNF4α and USF1 binding sites in OCT1 promoter. Further gene reporter experiments with the constructs in HepG2 cells helped to reveal the significant roles of the factors in OCT1 regulation.

Next, we generated two CYP3A4 gene reporter constructs with XREM and proximal regulatory regions of CYP34 gene and with both firefly and GFR reporter genes. Functional cellular experiments confirmed correct construction of the plasmids.

Finally, we generated chimera CAR expression construct with inserted alanine residue and EGFP, which confers low constitutive activity and no false translocation to nucleus. In fluorescent microscopy experiments, we confirm nuclear localization of CAR+A/EGFP protein after treatment with CAR ligand in HepG2 cells and activation of CYP3A4 luciferase gene reporter construct.

I can conclude that generated DNA constructs are functional and are valuable tools for further study of gene regulation.

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ALKALOIDS OF SELECTED *GALANTHUS, LEUCOJUM* AND *NARCISSUS* SPECIES AND THEIR BIOLOGICAL ACTIVITY

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More than 50% cases of dementia are nowadays caused byAlzheimer's disease (AD). AD is a progressive neurodegenerative disease and it causes gradual memory loss, disorientation and behavioral disorders which affect patient's social and occupational life. AD is characteristic by loss of neurons in some regions of brain – for example hippocampus and cortex. Ethiopathogenesis of this disease is not completely known – that is why the treatment is still just symptomatic. Formation of β-amyloid deposits in brain tissue plays an important role - it is a protein which creates extracellular plagues around neurites and causes their degeneration and death. Intracellular tangles are made up of the changed τ-protein. These tangles also cause death of the neuronal cell. The degeneration of neurons is supported by reactive oxygen radicals too. The another problem is a glutamatergic system disorder. This set of excitatory amino acids is important for correct long-term memory formation. Patients with AD suffer from glutamatergic system overactivation which leads to the formation of neuronal perturbation, excitotoxicity and apoptosis of neuronal cells. In patients with AD the acetylcholine (ACh) production is damaged. ACh is a neurotransmitter and its lack

participates in the development of AD. ACh is physiologically decomposed by enzyme acetylcholinesterase (AChE). The second enzyme taking part in ACh degradation is a butyrylcholinesterase (BuChE). In severe forms of AD, levels of AChE and choline acetyltransferase are decreased by as much as 90% compared with normal condition, while the concentration of BuChE increases. That's why the new inhibitors with dual enzymatic activity against AChE and also BuChE are sought.

Galanthus, Leucojum and *Narcissus* species belong to *Amaryllidaceae* family. Plants of this family produce wide range of specific chemical substances called *Amaryllidaceae* alkaloids. These alkaloids have various biological effects like anti-inflammatory, antivirotic, antineoplastic, antiparasitic, antimycotic and they are also able to inhibit erythrocytic AChE (HuAChE) and serum BuChE (HuBuChE).

Alkaloidal extracts of seven selected species and cultivars were analysed by GC/MS and alkaloids were identified from their mass spectra, retention times and retention indexes. Summary extracts were tested *in vitro* for their ability to inhibit HuAChE and HuBuChE using Ellman's method. Interesting inhibitory activities were shown by alkaloidal extracts of *Galanthus woronowii* ($IC_{50,HuAChE}$ = 8.65 ± 1.20 µg/mL), *Galanthus elwesii* ($IC_{50,HuAChE}$ = 10.29 ± 1.00 µg/mL), *Narcissus* cv. QUIRINUS ($IC_{50,HuAChE}$ = 17.72 ± 2.41 µg/mL) and *Narcissus* cv. VIRGINIA SUNRISE ($IC_{50,HuAChE}$ = 10.72 ± 0.83 µg/mL; $IC_{50,HuBuChE}$ = 29.62 ± 3.47 µg/mL) when galanthamine was used as a standard ($IC_{50,HuAChE}$ = 1.71 ± 0.007 µM; $IC_{50,HuBuChE}$ = 42.30 ± 1.30 µM).

Using preparative TLC (To : Et_2NH, 95 : 5) one alkaloid was isolated from alkaloidal extract of *Narcissus* cv. PROFESSOR EINSTEIN. The isolated compound was identified as a homolycorine and tested for its inhibitory activity against HuAChE (IC_{50} = 63.7 ± 4.3 µM), HuBuChE (IC_{50} = 151.0 ± 15.2 µM) and prolyloligopeptidase ($IC_{50,POP}$ = 173 ± 40.6 µM). Galanthamine and Z-Pro-prolinal ($IC_{50,POP} = 3.27 \cdot 10^{-3} \pm 0.02 \cdot 10^{-3}$ nM) were used as positive standards.

The study was supported by SVV 260 184.

CHANGES OF MPV DURING END-STAGE RENAL FAILURE: A LINK BETWEEN PLATELET SIZE, INFLAMMATION AND MAIN CAUSES OF CHRONIC KIDNEY DISEASE

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Mean platelet volume, a simple indicator of platelet size, is automatically calculated by blood analysers. Higher MPV refers to larger platelets, which are more reactive. These thrombocytes are assumed to have the greatest role in development of haemostatic disorders and the most of other cardiovascular diseases (CVD), which are the main cause of death in patients with end-stage renal failure[1]. The chronic renal failure (CRF) also supports a small permanent inflammation in the body[2] which can be measured by C-reactive protein. The aim of the study was to find out if there is a relation between MPV and CRP in patients undergoing a continuous renal replacement therapy (CRRP). We also focused on etiopathology of renal failure. The main causes of CRF all over the world are chronic glomerulonephritis, diabetic nephropathy, hypertension, polycystosis of kidneys and chronic interstitial nephritis[3]. We compared MPV values from three of these groups and tried to establish the correlation between pathogenesis of the disease and platelet activation.

A total of 102 patients who received the CRRP between November 2014 and February 2015 were taken into this retrospective study. The collected data included basic information such as gender, age, fundamental cause of renal failure and the length of dialysis program, if the transplantation of kidney has been realized and if the patient suffered from any cardiovascular disorders or the diabetes mellitus. Then MPV, platelet count and CRP taken from every first

week in these 4 months and finally some technical information such as the method of dialysis (hemofiltration, hemodialysis or hemodiafiltration and the regular regime of dialysis.

The sex ratio (M/F) of all patients was 11:6, the average age was 66,2 (23 - 91) years. 45,1 % of patients had already suffered from diabetes mellitus, 39,2 % had had some cardiovascular disease (ischemic heart disease, heart insufficiency, apoplexy etc.).

The mean MPV was 10,44 ± 0,96 which is closed to the upper limit of physiologic values (10,5 fl). The mean platelet count was 219 x 10^9/l and the mean CRP was 9,56 ± 10,70 mg/l. As predicted, the MPV values slightly diminished with increasing CRP (so with the higher level of inflammation), but the value of reliability of linear regression was very low (R^2 = 0,0013) that is why we could not consider it as a valuable confirmation of our theory.

The main causes of chronic renal failure were diabetic glomerulosclerosis (in 30 patients, 1st group), chronic interstitial nephritis (11, 2nd group), chronic glomerulonephritis (9, 3rd group) and other (62). The average value of MPV was the highest in the 1st group (10,79 fl) and the lowest in the 2nd group (10,57 fl).

We did not detect a significant relation between MPV and CRP, even if the trendline showed a negative correlation. The problem could be in interactions with other diseases the patients suffered, as CVD, hypertension and DM, which could influenced our values. The MPV was higher in patients with diabetic glomerulosclerosis. The other two groups could have lower MPV values due to an inflammatory etiology of the ailment.

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SYNTHESIS OF TETRAZOLE DERIVATIVES WITH HIGH ANTIMYCOBACTERIAL ACTIVITY AND THEIR INITIAL *IN VITRO* TOXICITY ASSESSMENT

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Tuberculosis is persistently considered to be one of the most dangerous and widespread infectious disease with estimated 1.5 million annual deaths worldwide (one third of entire population is infected). It is very specific illness with unique pathogenesis, progression and complicated treatment. The most frequent etiological agents of this disease are strains of *Mycobacterium tuberculosis*. Nowadays we can diagnose and cure this disease with success. On the other hand, it is still causing fatal issues in developing countries and is often found in immunosuppressed patients and people co-infected with HIV virus. Resistance to antituberculosis drugs has been rising constantly - the emergence of extensively drug resistance tuberculosis (XDR) has worsen the situation even more. From this reason a pharmaceutical laboratories all over the world are trying to develop new effective drugs that would provide faster and more effective treatment of this illness and prevent the forming and spreading of resistant strains.

The aim of this study was to synthetize three novel derivatives and subsequently to provide basic *in vitro* evaluation of their cytotoxicity (together with other substances from this series).

Cytotoxicity experiments were performed on 3T3 mouse nonmalignant fibroblast cell line. Cellular viability was assessed using Neutral Red uptake assay in 96-well plates. Epifluorescence microscopy was used for obtaining information of cellular and sub-cellular

morphology changes using CellMask Green (cytoplasmic membrane), Hoechst 33342 (nucleus) and MitoTracker Red FM (mitochondria) fluorescent probes. Additionally changes in mitochondrial inner membrane potential were monitored using JC-1 fluorescent probe.

Results of this study showed absence of inherent toxicity of the most of studied compounds up to their solubility limit in water-based media (up to 100 μM). Antimicrobial activities (MICs) of these substances are in ≈ 0.03-1 μM concentrations – this in combination with the low cytotoxicity render these substances as very promising antituberculosis agents.

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PHARMACOLOGICAL EVALUATION OF POTENTIAL ALZHEIMER´S DISEASE DRUGS

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Alzheimer´s disease (AD) is a neurodegenerative disorder affecting primarily ageing population. It is characterized by aggregates of amyloid plaque, neuofibrillary tangles of tau proteins and by loss of cholinergic neurons in the basal forebrain and hippocampus.[1] Cause of AD is still unknown and only symptomatic treatment is available thanks to acetylcholinesterase inhibitors (AChEI) and memantine. M_1 muscarinic positive allosteric modulators (PAMs) represent another variant of treatment that can improve cholinergic transmission. Thanks to their selectivity, they are able to decrease side effects.

The aim of the study was to measure novel compounds´ abilities to inhibit AChE and BChE and simultaneously act as M_1 PAM. Enzymes inhibition was measured spectrophotometrically (according to Ellman´s method) using 96 microwell plates and IC_{50} values were determined. The CHO cell line stably expressing the M_1 subtype mAChR was used for fluorescent measurement of compounds interaction with mAChR. Fluo-4 NW was used as fluorescent indicator, oxotremorine as an orthosteric agonist and BQCA (benzyl quinolone carboxylic acid) as a positive allosteric modulator. Statistical analysis of results was performed in GraphPad Prism6.

Unfortunately, none of the tested compounds acted as a PAM/allosteric agonist. All novel compounds acted as M_1 inhibitors. Moreover, AChE and BChE inhibition was comparable with standards.

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THE ENTRANCE OF INTRACELLULAR PATHOGEN *FRANCISELLA TULARENSIS* INTO B CELLS

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The main aim of the study was the analysis of entrance of intracellular pathogen *Francisella tularensis* into B lymphocyte.

Francisella tulerensis is intracellular gram-negative bacterium and it was chosen for its high virulence. It can be easily abused as a biological weapon.

The interaction with B cells is important, because a studies shown *F. tularensis* is able to induce an apopthosis in them. In the first part of the work we wanted to find out the influence of blocking receptors for entrance into cell on infection caused by *F.tularensis*.

It was detected by transmission electron microscopy and flow cytometry. We observed blocation on receptors CR1, CR2, CR3, CR4 a BCR. Next part of this was investigate effect of opsonization by antibody and complement system on the same infection. Their inhibitors were used for detection signal pathaways.

In the second part of this research we observed the cell fate – colocalization of bactery with endo-lysozomal markers. For the first part of work we used murine B lymphocytic cells line A20 and peritoneal B cells BALB/c. The colocalization was observed on the A20 cells only.

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SCREENING FOR CYTOTOXIC ACTIVITY OF AMARYLLIDACEAE ALKALOIDS

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Oncological diseases are one of the leading causes of death in the developed countries and the increase of its prevalence seems to be inevitable. According to World Health Organization´s International Agency for Research on Cancer (IARC) in Loyn, France, the incidence of cancer is expected to increase by more than 75% by the year 2030 in developed countries. In most cases oncological patients die due to resistance of cancer to therapy, metastasis and dissemination of cancer cells into vital organs. The standard treatment covers surgical intervention, radiotherapy and/or chemotherapy.

Additionally convential anticancer treatments damage healthy tissue, resulting in a variety of side effects. Therefore, substantial efforts are being inevested into identifying and developing compounds that would be able selectively target tumor cells while not damage healthy cells.

The search for new lead anticancer compounds is a crucial element of modern natural products research. Among various natural sources that have been investigated for constituents with potential use in cancer treatment, plants of the Amaryllidaceae family have been particularly promising and fruitful.

To date, about 50 of these alkaloids were tested against different cell lines. From these pilot studies we can conclude that the most active substances fall into free structural types: namely

lycorin, crinine and pancratistatin. Most of these active compounds were studied for IC_{50} values on diverse mammalian cells howewer mechanism of action remains to be determined.

In curent study we screened and determined IC_{50} *in vitro* growth inhibitory activity (using the MTT colorimetric assay) of 15 Amaryllidaceae alkaloids at concentrations up to 100µM in to cancer cell lines Caco-2 (human epithelial colorectal adenocarcinoma cells) and HT-29 (human colon adenocarcinoma cells) using the MTT colorimetric assay. A human normal intestine cell line (FHS-74int) was used as a control fot the overall toxicity. All tested alkaloids have been previously isolated in our laboratory from three plant species *Zephyrantes robusta*, *Chlidantus fragrans* and *Nerine bowdenii*.

Among the tested compounds lycorine, haemanthamine and haemanthidine exhibited the most potent cytotoxic potential against both tested cell lines, with IC_{50} values of 0,99 - 3,28µM for Caco-2 and IC_{50} 0,59 – 1,72µM for HT-29. Lycorine and haemanthamine showed only moderate toxicity against normal cells ($15\mu M < IC_{50} < 30\mu M$) in comparison to used standard vinorelbine, which is significant toxic to used normal cells (IC_{50} 3,98 ± 0,26µM). Other tested alkaloids showed moderate ($10\mu M < IC_{50} < 25\mu M$), weak ($25\mu M < IC_{50} < 100\mu M$) or no ($IC_{50} > 100\mu M$) cytotoxic potential against all tested cell lines.

Further step of the current study is the preparation of semisynthetic analogues by changing different parts of the structure of the most active compound haemanthamine. This compound is isolated in our laboratory in sufficient amount (10g) allowing such structure-activity relationship (SAR) study. The first series of haemanthamine analogues have been already synthetized. The prepared analogues are assayed for their cytotoxic activity.

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EXPRESSION OF DHRS8 AND DHRS12 ENZYMES IN HUMAN TISSUES

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Dehydrogenase/reductase (SDR family) member 8 (DHRS8, SDR16C2) and dehydrogenase/reductase (SDR family) member 12 (DHRS12, SDR40C1) are human microsomal enzymes belonging to the superfamily of short-chain dehydrogenases/reductases (SDR). This superfamily represents one of the largest protein groups. SDR enzymes participate in the metabolism of various xenobiotic and endogenous compounds and are involved in physiological and pathological processes[1,2]. However, there are still many enzymes which are only poorly characterised.

To this date, the expression on mRNA level and catalytic activity toward 5α-androstane-3α,17β-diol are the only available information about DHRS8[3,4]. Moreover, there is still no published information (apart from the prediction) regarding DHRS12.

The aim of this study was to examine the protein expression of DHRS8 and DHRS12 enzymes in various human tissues. The tissue samples were collected from five middle aged male subjects after the sudden death without apparent disease. Proteins of interest were detected using western blotting and specific antibodies. Recombinant form of searched proteins (DHRS12, DHRS8) expressed in sf9 insect cells was used as a control.

According to our results, DHRS8 is widely expressed in many tissues with the highest level in the liver and adrenal glands. On the other hand, the expression of DHRS12 was detected only in the brain. Our data could help to estimate the role of these enzymes in human body.

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