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Abstracts

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PXFL1

IN VITRO SCREENING OF STRUCTURALLY DIFFERENT TOPOISOMERASE II INHIBITORS FOR PREVENTION OF ANTHRACYCLINE CARDIOTOXICITY

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Anthracyclines (ANTs) retain the prominent role in many cancer treatments due to their high efficacy. However, the use of all of the ANTs is associated with a risk of severe cardiotoxicity. To date, dexrazoxane (DEX) has been the only cardioprotective agent approved for clinical use; therefore, it represents the main lead in the search for effective cardioprotection. The focus regarding its cardioprotective mechanism has recently shifted from metal chelation to its effect on topoisomerase II (TOP2).

This inspired us to examine various structural types of compounds described as TOP2 inhibitors for potential cardioprotective effect. In the first stage, we screened a series of commercially available compounds reported to inhibit TOP2 for their protective properties on primary cultures of neonatal rat cardiomyocytes. We also examined the effects of studied compounds on proliferation of HL-60, cell line derived from acute promyelocytic leukemia, and their effect on antiproliferative activity of daunorubicin. Because mitigation of adverse effect loses meaning if it diminishes the main effect.

From the series of inhibitors evaluated so far, three compounds show promising cardioprotection. Therefore, in the next stage, effect of the selected compounds on activity and depletion of TOP2 will be ascertained, to gain better mechanistical insight; and also, analogues of the perspective compounds are being prepared and studied.

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PXFL2

EVALUATION OF DAUNORUBICIN ANTIPROLIFERATIVE EFFECT ON TOPOISOMERASE 2B DEPLETED HL-60 GENERATED WITH CRISPR-CAS9

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Topoisomerase II β (TOP2B) inhibition was identified as one of the possible mechanisms of dexrazoxane protection of cardiomyocytes from anthracycline (ANT) cardiotoxicity. In 2012, Zhang et al. outlined that mice with heart specific depletion of TOP2B were prevented from ANT-induced cardiac damage.[1] Unlike heart cells, human leukemic cell line HL-60 contain both TOP2A and TOP2B isoforms. TOP2A enables cell division while the precise function of TOP2B was not fully understood yet. Dexrazoxane (DEX) as the only approved cardioprotective agent acts as catalytic inhibitor of both TOP2 isoforms, and due to its TOP2 inhibition effect is suspected of compromising the cytotoxicity effect of anthracyclines in cancer cells. The TOP2B was also implicated in the resistance of tumor cells and the increase of secondary malignances, we aimed to deplete TOP2B with CRISPR-Cas9 technology and evaluate the effects of daunorubicin (DAU) on these mutants regarding their sensitivity to DAU. HL-60 were transfected with specific CRISPR-Cas9 plasmid targeting TOP2B. Forty-eight hours after transfection the cells were sorted with GFP as a selection marker to 96-well plates. After approximately 6 weeks of cell growth individual clones were tested for TOP2B occurrence by immunofluorescence and western blotting. TOP2B deficient clones were spotted and genotyped to characterize individual clonal mutations. Based on these acquired data several homozygous and heterozygous TOP2B deficient mutants were identified. Antiproliferative effect of DAU was evaluated in both homozygous and heterozygous mutants using MTT.

The study was supported by SVV 260 416.

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PXFL3

TYROSINE KINASE INHIBITORS AS MULTITASKING SOLDIERS AGAINST CANCER DRUG RESISTANCE: THE EXEMPLARY CASE OF MIDOSTAURIN

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Tyrosine kinase inhibitors (TKIs) are proven active antileukemic agents that suppress aberrant tyrosine kinase signaling involved in cell proliferation. TKIs have shown additional targeting of proteins involved in cancer multidrug resistance, like membrane transporters and detoxifying enzymes. Midostaurin is a selective inhibitor of FMS-like tyrosine kinase-3 (FLT3) approved for the treatment of acute myeloid leukemia (AML), in combination with anthracycline daunorubicin (DAU). Midostaurin-based combination chemotherapy has demonstrated significant clinical benefits and safety, but the molecular mechanisms involved are still poorly understood. In this sense, there has been reported how carbonyl reducing enzymes (CREs) expressed in leukemic cells contribute to resistance towards daunorubicin. In this context, we evaluated the effect of midostaurin on DAU reduction by several recombinant CREs, observing a tight-binding inhibition of Aldo-keto reductase 1C3 (AKR1C3). Likewise, midostaurin decreased DAU metabolism in an HCT116 cell model overexpressing AKR1C3. Furthermore, acute myeloid leukemia cell line KG1a naturally expresses CREs that correlates to its inherent resistance to anthracyclines. Midostaurin performs a dual effect on KG1a cells, by inducing DAU accumulation but significantly reducing DAU metabolism. Confocal microscopy and flow cytometry showed that the combination with midostaurin increases the nuclear localization of daunorubicin in KG1a cells, probably due to the higher availability of the non-reduced form of DAU. Our findings revealed that midostaurin improves DAU cytotoxicity by the simultaneous inhibition of different proteins that are critical in cancer multidrug resistance.

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PXFL4

EMD1214063 REVERSES MULTIDRUG RESISTANCE BY INHIBITING THE EFFLUX FUNCTION OF ABCB1 AND ABCG2 TRANSPORTERS

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ABC (ATP-binding cassette) drug efflux transporters play an important role in pharmacokinetic drug-drug interactions as well as in the phenomenon of multidrug resistance (MDR) in cancer cells. EMD1214063 (EMD) is a novel c-MET tyrosine kinase inhibitor that has being developed for several types of cancer, including non-small cell lung cancer. In this study, we aimed to evaluate the inhibitory activity of EMD towards human ABC transporters and its role in the MDR. In accumulation studies in MDCKII cell lines overexpressing particular ABC transporters, we showed that EMD is an inhibitor of ABCB1 and ABCG2. Furthermore, we demonstrated that EMD1214063 significantly reverses ABCB1- and ABCG2-mediated daunorubicin and mitoxantrone MDR, respectively. For reversal experiments, MTT proliferation assay in MDCKII, A431 and HL60 cells overexpressing ABCB1 and ABCG2 transporters, was used. Additionally, EMD was found to be a substrate of ABCB1 but not of ABCG2 or ABCC1, in MDCKII monolayer transport assays followed by UHPLC/MS analysis. No significant induction effects of EMD on ABCB1, ABCG2 or ABCC1 mRNA levels were found in physiological cells as well as non-small cell lung cancer cellular models using qRT-PCR analysis. Overall, we conclude that EMD could participate in the pharmacokinetic drug-drug interactions and overcome the pharmacokinetic MDR phenomenon in cancer cells. Future in vivo confirmation of our results might potentially open the way for the establishment of safe and effective combination pharmacotherapy for many oncological patients.

The study was supported by the Czech Science Foundation (grant No. 20-20414Y), Grant Agency of Charles University (project No. 1568218/C) and finally by Charles University (SVV/2020/260-414).

PXFL5

MIDOSTAURIN AS A NOVEL MODULATOR OF ABC TRANSPORTERS IN ACUTE MYELOID LEUKEMIA

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Midostaurin is a multi-kinase inhibitor recently approved for the treatment of patients diagnosed with acute myeloid leukemia (AML) or myelodysplastic syndrome, who carry the FMS-like tyrosine kinase 3 (FLT3) mutation accountable for a poor prognosis. One of the most common mechanisms responsible for a failure of anticancer therapy is multidrug resistance (MDR) with ABC efflux transporters being one of important causative factors. Specifically ABCB1 and ABCG2 are confirmed to be related to resistant $CD34^+$ leukemic blast cells. In this study we aimed to evaluate interaction of midostaurin with ABC transporters using resistant HL60 cell lines and *ex vivo* isolated peripheral blood monocyte cells (PBMC) from patients *de novo* diagnosed with AML. Gene expression of ABC transporters was established in AML patients' PBMC employing droplet digital PCR. Our results showed that *ABCB1* and *ABCG2* were highly expressed in $CD34^+$ cells while differences between $FLT3^+$ and $FLT3^-$ patients fell short of statistical significance. Accumulation assays in resistant HL60-ABCB1 and HL60-ABCG2 cells revealed midostaurin as inhibitor of both transporters. When applied to PBMC of $CD34^+$ patients, midostaurin significantly increased the intracellular levels of mitoxantrone, a conventional anticancer drug that is recognized as a substrate of ABC transporters. Since a noticeable correlation of *ABCB1* and *ABCG2* expression with the effect of midostaurin on accumulation of mitoxantrone in PBMC was found, we can assume that the expression of ABC transporters might affect therapeutic outcomes of combination therapy in AML. In conclusion, we show here the potential of midostaurin to contribute to overcoming the pharmacokinetic MDR in AML patients and thereby prevent the pharmacotherapy failure.

The study was supported by SVV 260 414 and PRIMUS 20/MED/010.

PXFL6

OVERCOMING DAUNORUBICIN RESISTANCE MEDIATED BY ALDO-KETO REDUCTASE 1B10.

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Tyrosine kinase inhibitors (TKi) have been found to have effective antitumor activity and have been approved or are under clinical trials [1]. Recent studies show that some TKis are able to enhance the cytotoxicity of anthracyclines [2]. Dasatinib is an orally administered, small-molecule inhibitor of multiple tyrosine kinases that blocks the function of the Bcr-Abl protein that signals cancer cells to multiply. Targeted therapy of dasatinib is used to treat most cases of chronic myeloid leukemia and acute lymphoblastic leukemia in patients [3]. AKR1B10 has been recently found to be overexpressed in certain types of cancers, including hepatocellular carcinoma and lung cancer associated with tobacco smoking [4].

Our Combination strategy of the daunorubicin together with dasatinib may, therefore, minimize the adverse effects of each individual drug, enhance the effectiveness of the treatment and allow its prolonged continuity. Dasatinib exhibited a significant inhibitory effect on recombinant AKR1B10, with a half-maximal inhibitory concentration of 0.8 μM. Its inhibition constant Ki was found to be 0.4 μM, and the inhibition data best fitted a mixed-type mode with $\alpha = 1.7$. In conclusion, based on our results, dasatinib may affect the therapeutic efficacy of anthracyclines by preventing anthracycline resistance and reducing their adverse effects.

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PXFL7

INHIBITION OF HUMAN ALDO KETO REDUCTASE (AKR1C3) BY OLAPARIB – A POSSIBLE REMEDY FOR DAUNORUBICIN RESISTANCE

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The chemotherapeutic application of daunorubicin has significant drawbacks due to drug resistance and related cardiotoxicity. Several members of aldo-keto reductase and short-chain dehydrogenases/reductases superfamilies are responsible for reductive metabolism of parent drug to its less potent metabolite daunorubicinol and belong thus to the most important daunorubicin resistance drivers. Olaparib is a poly (ADP-ribose) polymerase inhibitor used in the treatment of patients with ovarian cancer. In this work, we have aimed to describe possible interactions of olaparib with selected daunorubicin reductases and evaluate their possible utilization for overcoming daunorubicin resistance. Results of incubation experiments with recombinant carbonyl reductases (AKR1C3, AKR1B10, AKR1B1, AKR1A1, and CBR1), showed significant inhibition of AKR1C3, the most active anthracycline reductase. High inhibitory potency (IC_{50} = 5.913 μM) was subsequently confirmed in intact HCT116 cells overexpressing AKR1C3. Using proliferation XTT assay in the same cellular model, we demonstrated the ability of olaparib to reverse enzyme-mediated daunorubicin resistance in a synergistic fashion. Currently, additional experiments focusing on the description of olaparib's effect on expression of *AKR1C3* gene in leukemic KG1α and hepatic HepG2 cells are being conducted. In conclusion, our results present olaparib as a potent AKR1C3 inhibitor able to effectively attenuate daunorubicin resistance at clinically relevant concentrations. Future *in vivo* studies would be helpful to support the rationality of our conclusions and possibly offer new therapeutic option for oncological patients.

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PXFL8

NOVEL PHOTODYNAMICALLY ACTIVE HYDROPHILIC AND AMPHIPHILIC ANIONIC (AZA)PHTHALOCYANINE DERIVATIVES FOR TREATMENT OF TUMOROUS DISEASES

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Photodynamic therapy is a clinically approved non-invasive treatment and subject of intense research for the eradication of solid tumors. Phthalocyanines (Pcs) proved to be very promising photosensitizers (PS). Aim of this work is to evaluate the photodynamic activity of newly synthesized anionic water-soluble zinc(II) Pcs with sulfonyl or carboxyl substituents in *in vitro* conditions. Cytotoxicity experiments were performed mainly on human cervix carcinoma cell line HeLa using neutral red uptake assay. Localization of the compounds within the cell, uptake profiles of PSs to the cells and morphological changes after irradiation were also studied. The results of individual experiments have shown high photodynamic activity after irradiation (phototoxicity; EC_{50}) and exceptionally low inherent toxicity (toxicity in the absence of activating light; TC_{50}) of all studied compounds. Phototoxicity was further evaluated on two other human tumor cell lines: MCF-7 (breast carcinoma) and HCT116 (colorectal carcinoma). The most suitable properties were achieved with P44 (EC_{50} = 0.33 μM, TC_{50} > 1000 μM) in the serum-containing medium. For all studied compounds, photodynamic effect resulted in significant morphological changes indicating ongoing cell death. It is worth nothing that photodynamic activity of all studied compounds is negatively affected by the presence of serum (serum-free conditions resulted in up to 95-time increase in photoxicity). Based on obtained results, selected compounds will be included in subsequent studies on 3D spheroid cultures as well as in the *in vivo* evaluation of their photodynamic efficiency on mouse tumor model.

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PXFL9

PRECISION-CUT INTESTINAL SLICES FROM HUMAN TISSUE AS AN *EX VIVO* MODEL FOR ABCB1 TRANSPORTER INDUCTION

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P-glycoprotein (ABCB1), an ATP-binding-cassette efflux transporter, limits intestinal absorption of its substrates and is a common site of drug-drug interactions (DDIs). The drugs causing DDIs on this transporter can act as substrates, inhibitors and/or inductors. Therefore, the absorption of the compounds may be changed and can lead to inappropriate drug plasma levels. Current options for investigating the induction process are limited. For this purpose, we decided to evaluate and optimise human precision-cut intestinal slices (PCIS) to be suitable for long term induction studies. 3 types of media were evaluated: I) Williams' Medium E (WME) as a standard medium used for PCIS, II) two different organoid media (ORG and Vacy) with cooperation of Applied University of Utrecht. Incubations were performed with and without Rifampicin, known inducer of the ABCB1, to validate the model for induction studies

During the evaluation, we collected samples after 24, 48, 72h. ATP/protein measurement was used as a viability marker, RT-PCR and immunohistochemical methods were used to study ABCB1 levels. Rhodamine123 accumulation assay was used as a functional control of ABCB1 expression. In WME after 24h Rifampicin induced the efflux activity of the ABCB1 and increased level of the *ABCB1* mRNA. With increasing incubation time, we saw a similar effect, but also with the increasing deviation. On the other hand, Vacy and ORG medium have a better effect on the ATP/protein level, proliferation and the stability of the activity of ABCB1, but we didn’t observe any effect on the induction with added Rifampicin.

Finally, we could summarize that human PCIS can be used as an *ex vivo* induction model of the ABCB1 in the WME medium. The effect is observed on the activity and mRNA level.

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PXFL10

IN VITRO AND *EX VIVO* EVALUATION OF ANTI-HIV AND ANTI-HCV DRUGS EFFECTS ON DIGOXIN INTESTINAL ABSORPTION

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Efflux transporter P-glycoprotein (ABCB1) is known to limit intestinal absorption of its substrates and represents a site of drug-drug interactions (DDIs). Competition on specific ABCB1 binding sites (for rhodamine123, Hoechst33342, and digoxin) may result in increased drug bioavailability. Using *in vitro* bi-directional transport studies across Caco-2 cells and *ex vivo* method of accumulation in rat- and human-derived precision-cut intestinal slices (PCIS) we showed in previous study that numerous anti-HIV and anti-HCV drugs reveal ability to increase absorption of model ABCB1 substrate, rhodamine123. In this follow-up project, we aimed to investigate the potency of anti-HIV and anti-HCV drugs to decrease intestinal ABCB1-controlled efflux of digoxin and thus to bring direct evidence about the molecular mechanism of interactions between antivirals and digoxin in the intestinal barrier. We found that lopinavir (50 μM), ritonavir (100 μM), atazanavir (50 μM), darunavir (50 μM, 100 μM), daclatasvir (20 μM), grazoprevir (20 μM, 50 μM) and asunaprevir (20 μM, 50 μM) inhibit the efflux of digoxin in Caco-2 cells and human-derived PCIS, while possible interindividual variability was observed in the inhibition of intestinal ABCB1 by rilpivirine (20 μM), abacavir (100 μM), elbasvir (5 μM) and velpatasvir (5 μM). Abacavir (100 μM), dolutegravir (10 μM), elbasvir (5 μM), velpatasvir (5 μM) and sofosbuvir (100 μM) revealed no inhibition of intestinal ABCB1 in Caco-2 cells. In conclusion, we have demonstrated that tested antivirals have potency for DDIs on intestinal ABCB1 with drugs with affinity to digoxin binding site. Our data contribute to explaining the molecular mechanism of reported increased bioavailability of drugs interacting with digoxin binding site when administered together with antivirals.

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PXFL11

EFFECT OF ANTIDEPRESSANTS ON PLACENTAL HANDLING OF SEROTONIN

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Serotonin (5-HT) is a crucial monoamine for proper fetal development/programming and, therefore, tight regulation of 5-HT homeostasis in the feto-placental unit is required throughout gestation. It is suggested that early in pregnancy the placenta supplies 5-HT to the embryo/fetus. However, at later stages of gestation, the fetus can produce its own 5-HT from maternally-derived tryptophan. Recently, we have discovered a new pathway of placental serotonin handling: placental extraction of excess 5-HT from fetal circulation through organic cation transporter 3 (OCT3). This transporter is known to be inhibited by many endogenous compounds and pharmacological agents including antidepressant drugs. Up to 25% of pregnant woman suffer from depression and approximately 10% are using antidepressants (ADs), typically serotonin-reuptake inhibitors. Safety of this treatment is still discussed, since many adverse effects have been reported. We hypothesize, that ADs can affect serotonin handling in placenta via inhibition of SERT and/or OCT3. This inhibition could lead to accumulation of 5-HT in placental circulation. Experiments were performed using *in situ* dually perfused rat term placenta and *ex vivo* membrane vesicles isolated from human term placenta. Six ADs were tested (paroxetine, citalopram, fluoxetine, fluvoxamine, sertraline, venlafaxine) to affect 5-HT uptake by placenta. We observed significant inhibitory effect by all ADs in both human and rat placenta. We suggest that AD use in pregnancy may affect placental homeostasis of 5-HT and, therefore, placental and/or fetal development.

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PXFL12

EFFECT OF GESTATION AGE ON EXPRESSION AND FUNCTION OF ENZYMES INVOLVED IN TRYPTOPHAN METABOLISM IN HUMAN AND RAT PLACENTA

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Tryptophan (TRP) is an essential amino acid that, besides being utilized for protein synthesis, is a precursor of key physiological biomolecules, such as kynurenine (immunosuppressive), quinolinic acid (neuroactive), serotonin and melatonin. In the placenta, TRP is metabolized via the serotonin (5-HT) and kynurenine (KYN) pathways and the relative flux of substances through these pathways changes depending on the physiological state. We hypothesize that embryo and fetal needs of TRP and its metabolites change over the course of gestation. Therefore, in our study, we investigated gestational age-dependent changes (expression/function) of key enzymes and transporters in tryptophan metabolism by KYN and 5-HT pathways in both human and rat placenta. In detail, we analyzed gene and protein expression of 5-HT transporters (SERT and OCT3) and gene/protein expression and function of TRP metabolizing enzymes (TPH1,2, MAO-A, IDO1,2) in first and third trimester human placenta and in rat placenta of gestation ages 15, 18 and 21. In both human and rat placenta, we detected significant effect of gestation age on expression and/or function of investigated proteins. We suggest these regulatory pathways control levels of TRP and 5-HT in feto-placental unit to ensure proper embryo and fetal development throughout pregnancy.

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PXFL13

THE CONTRIBUTIVE ROLE OF THE PLACENTAL ABCC1 IN MATERNAL AND FETAL PROTECTION. THE CASE OF MARAVIROC

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The protection of the developing fetus against potentially toxic xenobiotics is traditionally discussed in the context of efflux activity of ATP-dependent (ABC) drug transporters, especially ABCB1 and ABCG2, which are located in the apical membrane of the maternal blood facing trophoblast layer. CCR5 antagonist maraviroc shows low cord blood-maternal blood ratio (0.33), indicating limited transfer across placenta. Based on our previous study this could be attributed to the maraviroc substrate affinity toward ABCB1. Nevertheless, only negligible contribution of ABCB1 was recorded in closed-circuit dual perfusion of maraviroc across human placental cotyledon. Surprisingly, we observed decline of maraviroc concentration from circulating perfusates in both, materno-fetal and feto-maternal directions, suggesting retention of maraviroc in placental tissue. Subsequent *in vitro* studies revealed transport of maraviroc with ABCC1 transporter. Localization and function of placental ABCC1 is an object of many discussions, nevertheless, it is obviously placed in basal membrane of trophoblast layer. Besides the trophoblast, we show also high expression of *ABCC1* mRNA in the fetal endothelial cells. Considering placental structure, ABCC1 localization indicates possible transport of its substrates from both maternal and fetal circulations and their accumulation in placental interstitium, which corresponds with the situation observed during perfusion of maraviroc. Since placenta is a temporary organ leaving the body after delivery, we hypothesize ABCC1 might contribute to accumulation of xenobiotics in the placental tissue, as a possible part of a complex strategy ensuring protection of mother and fetus during pregnancy.

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PXFL14

PRIMARY HUMAN HEPATOCYTE SPHEROIDS AS *IN VITRO* PLATFORM FOR PRECLINICAL DRUG DEVELOPMENT

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Primary human hepatocytes are considered as a golden standard of *in vitro* models for evaluating hepatic metabolism and toxicity during drug development. However, a culture of primary human hepatocytes is known to lose hepatic phenotype resulting in a significant widespread alteration of metabolic capacity.

Hepatocyte dedifferentiation occurs due to loss of a structural architecture as hepatocytes are disrupted from their organized microenvironment and seeded in a monolayer culture. In this line, the growing effort has been put to develop more relevant *in vitro* models closely mimicking physiological cell environment and phenotype.

Recently, 3D spheroids of primary human hepatocytes have been developed using low adherent plates and well-defined culture conditions. This model showed similar molecular phenotype as that present in adult human liver and maintained hepatocyte functions over the long term.

In my talk, I will focus on the application of 3D hepatocytes in assessing of preclinical pharmacokinetic and toxicological properties of drug candidates.

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PXFL15

CHARACTERIZATION OF TRANSCRIPTOME IN HUMANIZED CAR MICE REGULATETED BY MODEL MURINE LIGAND TCPOBOP

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Nuclear receptor CAR (constitutive androstane receptor; Nr1i3) is a hepatic regulator of xenobiotic and endobiotic metabolism.[1] In mice, CAR activation normalizes glucose and triglyceride serum levels and decreases body weight during high fat diet and nutritional stress.[2] Murine CAR agonist, 1,4-bis[2-(3,5 dichloropyridyloxy)] benzene (TCPOBOP) is connected with hepatomegaly and hepatic proliferation.[3] We studied the effect of TCPOBOP in the wild type mice as well as in mice with humanized CAR, which is not activated by TCPOBOP. We found that TCPOBOP increases liver weights in the both genetic backgrounds independently on CAR activation. Gene expression study shows similar trends on proliferation genes after TCPOBOP treatment, when metabolism is strictly regulated in CAR-dependent manner. Our data shows that TCPOBOP may be a non-specific ligand promoting hepatocyte proliferation independently of human CAR activation.

The study was supported by GAUK 170-050/75006.

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PXFL16

DISCOVERY OF POTENT GPBAR1 AGONISTS/FXR ANTAGONISTS

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Bile acid receptors, GPBAR1 and farnesoid X (FXR), emerge as important regulators of glucose, lipid and steroids metabolism in the intestine and liver. Recent animal experiments showed that compounds with combined GPBAR1 agonistic activity in the intestine and FXR antagonistic activity in the liver may be promising candidates for the treatment of glucose metabolic diseases. In the study, we have discovered first-in-class GPBAR1 agonist/FXR antagonists based on testing a set of steroid molecules derived from obeticholic acid (OCA), the prototype FXR agonist. Several derivatives demonstrated potent activation of TGR5, among them compound 2 appeared as highly potent GPBAR1 agonist with EC_{50} being 12 nM in cellular reporter assays. At the same time, compound 2 as well as compound 8 inhibited FXR activation in various FXR reporter gene assays and suppressed OCA- and GW4064-mediated regulation of FXR target genes in differentiated HepaRG cells and primary human hepatocytes. In conclusion, we discovered novel class of dual GPBAR1 agonists/FXR antagonists

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MATHEMATICAL MODELING OF THE SIGNALING PATHWAYS OF NUCLEAR RECEPTORS

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The talk is focusing on the mathematical modeling on the signaling pathways of obeticholic acid activated farnesoid X receptor (FXR). Nuclear receptors like FXR are a family of ligand-regulated transcription factors that are activated by lipid-soluble substances. These substances cross the plasma membrane and interact directly with the nuclear receptors inside the cell.[1] Ordinary differential equations (ODEs) will be used to describe the gene transcription products changes over time. The final model will be used for predictions and extrapolations. FXR does not directly bind to the CY7A1 promoter. Rather, FXR induces expression of small heterodimer partner (SHP), which then functions to inhibit transcription of the CYP7A1 gene.[2] This process is modeled as a continuous function of time: $dR/dt=Kin-(1-(Imax*C^n/(IC50^n+C^n)))-Kout*R$ where drug effect is mediated as inhibition of SHP. Graphical comparison of observations and simulated predictions depicted a good fit of our model to the dataset. Additional mathematical models that describe and link these pathways will be built.

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PXFL18

PROBING THE STRUCTURE AND FUNCTION OF THE CYTOSOLIC DOMAIN OF THE HUMAN ZINC TRANSPORTER ZNT8 WITH NICKEL (II) IONS

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The human zinc transporter ZnT8 is important for assembly of insulin hexamers of β-cells with zinc and for its storage. Its structure and function is modelled on the basis of the 3D structure of the *E.coli* zinc exporter YiiP[1]. However, there are important differences in function as the YiiP protein exports an excess of zinc from cells, whereas ZnT8 exports zinc into subcellular vesicles when there is no apparent excess of zinc. There are two variants, one with tryptophan (W) and the other one with arginine (R) at position 325. These variants have generated considerable interest as the R-variant is associated with a higher risk of developing type 2 diabetes[2]. Since these mutations are at the apex of the C-terminal domain (CTD) towards the cytoplasm, it is not clear how they would affect zinc transport. We expressed the CTD of both variants of human ZnT8 and have begun structural and functional studies. In particular, we found that (i) the metal binding site of the human protein is different from that of *E.coli* protein, (ii) the human protein has a C-terminal extension with three cysteine residues that bind also zinc, (iii) there are small differences in stability between the two variants, and (iiii) nickel ions bind to the cytoplasmic domain of the zinc transporter ZnT8.

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PXFL19

ISOLATED SILYMARIN FLAVONOLIGNANS AND THEIR ABILITY TO INTERACT WITH TRANSITION METALS AND PLATELETS

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Silymarin, a complex of flavonolignans extracted from fruits of *Silybum marianum* (L.) Gaertn., is approved in the EU as a drug. It is also frequently used as a food supplement. Flavonolignans have a polyhydroxylated structure and are poorly absorbed. In addition, if absorbed, they are rapidly conjugated. For this reason, parent flavonolignans rather remain in the gastrointestinal tract while their conjugates are the dominant forms in the systemic circulation. This study was focused on testing 1) the ability of optically pure flavonolignans to interact with transition metals and 2) if their sulfates can block platelet aggregation. Only 2,3-dehydrosilybin (racemate as well as both enantiomers – A and B) has shown moderate ability to chelate iron and copper. Silybin A, silybin B and silychristin were less potent or inactive chelators. Silychristin was found to be the most potent iron and copper reductant. This study also discovered a low potential of sulfates of flavonolignans to block aggregation in whole human blood. Parent flavonolignans were tested for comparison, but their potential was low as well, since it was observed only at concentrations ≥ 120 μM. Mechanistic study showed that their mild activity was likely mediated by antagonism at thromboxane receptors. Although some silymarin flavonolignans blocked recombinant cyclooxygenase 1, their effect on this platelet enzyme in whole human blood was negligible. In conclusion, it is highly improbable that this activity would be manifested *in vivo* due to relatively high concentrations needed to evoke this effect. Contrarily, oral administration of silymarin may influence the kinetics of copper and iron in the GIT.

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PXFL20

PLATELET AGGREGATION IN HEALTHY POPULATION: PRELIMINARY DATA ON AGE-DEPENDENT DIFFERENCES

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Primary haemostasis is a process which contributes to preserving the integrity of the cardiovascular system. It is enabled by platelets, anucleate cells circulating in the bloodstream which aggregate in response to certain stimuli. Altered platelet aggregation can severely affect circulation and lead to many cardiovascular diseases. Dysregulation of this process can result in life-threatening events, such as stroke and acute myocardial infarction, which are the most prevalent causes of mortality in developed countries[1]. An important factor influencing platelet aggregation is the age. There is, however, little data concerning the significance of this factor in experiments using whole blood. Therefore, we have performed a screening of 11 healthy individuals aged 21-58 of both sexes using Multiplate analyzer, which utilizes whole blood and allows examination of various aggregation inducers. A response to several aggregation inducers and standard drugs affecting these inducers were evaluated. We have observed a fairly high interindividual variance with all inducers and used drugs. Preliminary data showed a decrease in response to adenosine diphosphate (ADP) with increasing age. Younger individuals also appeared to be more responsive to acetylsalicylic acid (ASA). Authors are aware that these are initial data and a higher sample size is required to reach more solid conclusion.

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PXFL21

SOLUBLE ENDOGLIN, DOES NOT AFFECT CHOLESTEROL AND BILE ACIDS METABOLISM IN NASH MOUSE MODEL

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Plasma concentrations of soluble endoglin (sEng) are increased in cardiovascular and metabolic diseases associated with hypercholesterolemia, which affect liver functions. Previously, we showed that high sEng plasma levels affect cholesterol and BA homeostasis based on complex liver and intestinal effects. Therefore, the aim of the present study was to investigate effects of high levels of sEng on cholesterol and BA metabolism in liver upon induction of non-alcoholic steatohepatitis (NASH). Three-months-old wild-type and transgenic male mice overexpressing human sEng were fed for 6 months with high fat diet enriched with cholesterol and fructose or chow diet and underwent *in vivo* study with plasma and bile collection. Plasma biochemical analysis, LC/MS of plasma BA and histology were performed. Expression of enzymes and transporters in liver were assessed by qRT-PCR and Western blot. HFD significantly increased body and liver weight, and analysis of liver tissue confirmed NASH by presence of steatosis, fibrosis, oxidative stress, increased activity of ALP and ALT, and hypercholesterolemia in both HFD groups. However, high sEng levels did not significantly modulate development of diet-induced NASH and associated changes in cholesterol and BA metabolism in mice.

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PXFL22

ENDOGLIN MODULATES ADHESION AND TRANSMIGRATION OF MONOCYTES IN OXYSTEROL INDUCED ENDOTHELIAL DYSFUNCTION

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Endoglin (CD105, TGF-βRIII receptor), is essential for proper function of endothelium, but also participate in inflammatory infiltration of monocytes. We hypothesized that endoglin play crucial role in monocyte adhesion and transmigration via endothelial cells when exposed to oxysterol simulating oxidized LDL effects in atherogenesis. HAECs were exposed to 7K (5, 10ug/mL) for 12 hours. Gene expression (endoglin, KLF6, RELA (NF-κB p65), NR1H3 (LXR), ICAM-1) was evaluated using qRT-PCR. Protein levels of endoglin, ICAM-1 and P/E-selectins were evaluated by flow cytometry analysis. Protein levels and localization of RELA, eNOS, p-eNOS was evaluated using confocal fluorescent microscopy. Gene expression and protein levels of endoglin, eNOS, p-eNOS and cell adhesion molecules (ICAM-1, E/P-selectin) as well as transcription genes regulating endoglin expression were significantly increased after premedication with 7K compared to non-treated cells. Inhibition of transcription factors (KLF6, RELA, NR1H3 resulted in inhibition of 7K induced increase of endoglin expression. 7K was able to increase adhesion and transmigration of THP-1 monocytes, trough endothelial cells monolayer. Silencing of endoglin in HAECs inhibited adhesion and transmigration of THP-1 monocytes. In this study, we demonstrated that 7K is able to induce inflammation and increase endoglin expression in endothelial cells via activation of KLF6, RELA and NR1H3 transcription genes. Moreover, we showed that 7K induced adhesion and transmigration of monocytes trough endothelial monolayer depends on the expression of endoglin suggesting that endoglin might play crucial role in cholesterol (oxysterol) induced endothelial dysfunction.

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PXFL23

ENDOGLIN EXPRESSION, SIGNALIZATION AND FUNCTION IN IFLAMMATION INDUCED ENDOTHELIAL DYSFUNCTION

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Endoglin (Eng) expression is linked to regulation of endothelial nitric oxide synthase (eNOS) in endothelial cells resulting in proper function of endothelium. On the other hand, it was proposed that Eng also participates in inflammatory infiltration of leukocytes through endothelium and thus plays negative role in endothelial dysfunction. We aimed to evaluate Eng expression, signalization and function related to endothelial dysfunction induced by proinflammatory tumour necrosis factor alpha (TNF-α) in human aortic endothelial cells (HAECs). HAECs were treated with 10ng/mL TNF-α for 12 h. The mRNA expression of Eng, eNOS, adhesion molecules (ICAM-1, VCAM-1) and transcription factors (KLF6, NF-κB p65 and LXR-α) was measured with qRT-PCR. Protein levels of membrane Eng, ICAM-1, VCAM-1, P/E-selectin and MMP-14 were measured by flow cytometry and soluble endoglin (sEng) levels by ELISA. Transmigration assay was performed using Nunc cell culture inserts. TNF-α treatment decreased mRNA expression and protein levels of Eng and eNOS. The mRNA expression and protein levels of cell adhesion molecules and MMP-14 were significantly increased as well as sEng levels. Interestingly, meanwhile mRNA expression of KLF6 and NF-κB were increased; mRNA expression of LXR-α was decreased. TNF-α induced inflammation led to increased adhesion but not transmigration of monocytes through endothelial cells. We demonstrated that inflammation decreases endoglin expression, increases adhesion but does not affect transmigration of monocytes through aortic endothelial cells. Reduced expression of Eng and increased levels of sEng (that inhibits effects of membrane endoglin) might be responsible for no change in transmigration of monocytes under inflammatory conditions. We propose that Eng participates on the regulation of endothelial dysfunction during inflammation, but to which extent must be further investigated.

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PXFL24

THE TOXICITY OF ALANTOLACTONE AND GERMACRONE TOWARDS DIFFERENTIATED HEPARG CELLS AND THEIR INFLUENCE ON CHOLESTEROL METABOLISM

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Sesquiterpenes alantolactone (ALA) and germacrone (GER) are naturally occuring molecules that are being studied as potential anti-cancer agents. ALA is one of the major sesquiterpene lactone compounds isolated from the roots of *Inula helenium*, *Asteraceae*. GER is a main bioactive constituent found in *Zedoary* oil product, which is extracted from *Curcuma zedoaria* Roscoe, *Zingiberaceae*. Both of these plants have been used in traditional medicine historically. Using the differentiated HepaRG (dHepaRG) cells, a human hepatocyte-like model, we wanted to compare the toxicity towards dHepaRG cells in comparison with results published on highly proliferative cancer cell lines after ALA and GER treatment. Furthermore, a bioinformatic tool BATMAN-TCM [1] was searched for new molecular targets of tested sesquiterpenes. Analysis of their common targets lead us to studying their effects on cholesterol metabolism and 3-Hydroxy-3-Methylglutaryl-CoA Reductase (HMGCR), a major regulatory enzyme in mevalonate pathway. HMGCR protein and mRNA expression were studied at multiple time points, concentrations, as well as single and multiple dose. HMGCR protein expression has shown inhibition after ALA and GER treatment, but mostly at the highest concentrations tested, equal to respective half-maximal inhibitory concentration of cell viability. The mRNA changes were much more variable and time and concentration dependent. The cholesterol level in dHepaRG cells was measured by Amplex Red Cholesterol Assay Kit in a multiple dose experiment in comparison to the model inhibitor lovastatin.

The study was supported by the Czech Science Foundation (Grant No. 18-09946S).

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PXFL25

THE UDP-GLYCOSYLTRANSFERASES IN *HAEMONCHUS CONTORTUS* AND THE METABOLISM OF ANTHELMINTICS

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UDP-glycosyltransferases (UGTs) are important enzymes in the metabolism of xenobiotics and eobiotics. Glycosylation is often the most important conjugation reaction catalyzed by these enzymes in drug metabolism. This reaction results in enhanced elimination of the drug from the organism. Increased expression of UGTs can cause reduction of pharmacotherapy efficiency and development of resistance to drugs. Our model organism is *Haemonchus contortus* a gastrointestinal parasite of small ruminants that have a great ability to develop resistance to anthelmintic drugs. Our previous metabolism study showed that albendazole, ricobendazole and flubendazole underwent several glycosylation steps. Differences of glycosides quantities between resistant and sensitive strains confirmed the connection between anthelmintics metabolism and resistance.[1] In addition, some enzymes from the UGT superfamily, e.g. UGT368B2, are significantly more expressed in adult *H. contortus* of resistant strains than sensitive strains.[2] For functional characterization, the UGT368B2 was expressed in baculovirus-infected insect cells. However, the preliminary results show that UGT368B2 cannot metabolize benzimidazole anthelmintics but steroids. This particular UGT has different role in the organism than biotransformation of xenobiotics (e.g. benzimidazoles). Revealing the features of UGTs from *H. contortus* (e.g. affinity to hexose or to different substrate) could contribute to a more detailed understanding of the reaction's mechanism catalyzed by these enzymes and their role in helminths.

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PXFL26

PROFILING MICRORNA EXPRESSION IN SUSCEPTIBLE AND RESISTANT STRAINS OF *HAEMONCHUS CONTORTUS* USING SMALL RNA SEQUENCING

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The widespread development of anthelmintic resistance (AR) in *Haemonchus contortus* compromises treatment of helminthosis caused by this economically important parasite of small ruminants. The molecular mechanisms of AR are not fully elucidated. In our study, we focus on microRNAs (miRNAs) which are a class of small non-coding RNAs. MiRNAs play important role in post-transcriptional regulation of gene expression and their dysregulation has been linked to a range of different pathologies. A comprehensive understanding of the functions of miRNAs in AR might help us develop better strategies to the sustainable parasite control. For this reason, we undertook the small RNA sequencing of *H. contortus* isolates with various level of resistance to anthelmintics, namely the susceptible strain, the benzimidazole resistant strain and the multi-drug resistant strain. Differential expression analysis revealed significantly up- or down- regulated miRNAs in adults of resistant strains in comparison to sensitive ones. Since cytochromes P450, UDP-glycosyltransferases and P-glycoproteins were reported to play role in drug resistance[1], we investigated them as the putative targets of differentially expressed miRNAs using RNAhybrid software. Moreover, from the sequencing data, 207 sequences were defined as potential novel miRNAs using miRDeep2 program.

The study was supported by Charles University (PRIMUS/17/SCI/4).

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PXFL27

METABOLIC PATHWAYS OF NEW POTENTIAL ANTHELMINTICS IN *HAEMONCHUS CONTORTUS* AND ITS HOST

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Haemonchus contortus is one of the most important parasitic nematodes of small ruminants with worldwide distribution causing significant loses to many farmers. Anthelmintic drugs still represent the main strategy to control burdens of *H. contortus*. Unfortunately, wide spread resistance to available anthelmintics makes treatment difficult. Therefore, there is a global need for new and effective anthelmintic drugs. Two newly synthetized compounds HBK4 and BLK127, and already registered antipsychotic drug sertraline are promising candidates of new anthelmintics. It is known, that drug resistance is associated with accelerated drug metabolism and for this reason we would like to compare biotransformation of HBK4, BLK127 and sertraline in drug-resistant and drug-sensitive strains of *H. contortus.* In addition, we will also monitor biotransformation and hepatotoxicity of these compounds in ovine liver.

The study was supported by Charles University Grant Agency (1568519).

PXFL28

CIRCULATION OF ANTHELMINTICS IN THE ENVIRONMENT

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Several ways can cause drug resistance in sheep breeding. Our project monitors the intake of a residual amount of the albendazole (ABZ) and its transformation products (TPs) during grazing of sheep.

Our preceding studies showed the ability of plants to uptake and metabolize anthelmintics such as benzimidazole, macrocyclic lactones, and amino-acetonitrile derivatives in six plant species. Plant derivated metabolites can be considered as deactivation products but some of them are even more anthelmintically effective than the parent compound. Moreover, a lot of anthelmintics metabolites (especially glycosides) can be converted back to parent compound through enzymatic or acidic hydrolysis in the gastrointestinal tract of grazing animals. In the case of infected animals, nematodes thus might be exposed to very low doses of anthelmintics and this phenomenon could help to increases the anthelmintic resistance.

This project simulates such a situation and monitors the occurrence of residues of ABZ and TPs in biological samples collected from ten domestic sheep (*Ovis aries*). In the pilot study naive sheep (e.g. without nematodes) were used. Two species of meadow plants *Medicago sativa* and *Trifolium pratense*, common plants on pastures, were chosen. An experimental field area with these plants was fertilized by excrements from ABZ treated sheep (different flock of sheep) in spring 2019.

ABZ and TPs have been found in the fertilized plants and in related soil too, after two months from fertilization. Furthermore, these plants were administered to the sheep for ten days and during that time samples of abomasum contents, faeces, and plasma were collected in different time intervals. ABZ and the main TPs, ABZ-sulfone, and ABZ-sulfoxide, were detected in all samples. UHPLC-MS/MS was used for qualitative and quantitative analyses.

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PXFL29

EFFECT OF ANTHELMINTIC RESIDUES ON TRANSCRIPTION LEVEL OF BIOTRANSFORMATION ENZYMES

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Anthelmintics, the only effective treatment of devastating infection diseases of animals caused by parasitic helminths, have been widely and heavily distributed all over the world. In the agriculture industry the most used classes of anthelmintics are benzimidazoles, macrocyclic lactones, imidazothiazoles and amino-acetonitrile derivatives. Parasitic helminths, specially nematodes, have developed resistance to all of them. In front of all parasites stands *Haemonchus contortus*, as the quickest nematode in resistance development. This hematophagous parasite living in sheep abomasum causing enormous losses in animals' production is well studied for mechanisms of resistance development. *H. contortus* prosper with very effective detoxification system via biotransformation enzymes and can relatively quickly react to constant usage of anthelmintic drugs. In our current studies we observed that even very low – sublethal concentrations of anthelmintics, that may be preserved in environment, can cause up and down regulation of biotransformation enzymes on transcription levels. Furthermore, the analysis of albendazole metabolites showed enhanced biotransformation after preincubation in sublethal concentrations of albendazole.

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PXFL30

PRELIMINARY RESULTS OF ANTI-INFLAMMATORY ACTIVITY IN SELECTED FERN SPECIES

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Ferns are a potential source of bioactive phytochemicals and based on our previous research, we tested selected species for the anti-inflammatory activity. The crude methanol extracts were screened at a concentration of 10 µg·mL-1 for inhibitory activity against pro-inflammatory enzymes cyclooxygenases (COX-1 and COX-2) and 5-lipoxygenase (5-LOX). The COX-1 inhibitors are reported as effective in the prevention of neuroinflammatory or cardiovascular diseases1, and several tumours2. COX-2 is considered as a key pro-inflammatory enzyme, over-expressed in most sites of inflammation and responsible for the characteristic inflammatory symptoms (redness, pain, edema, fever and loss of functions)2. 5-LOX inhibitors are reported to play a role in the prevention of tumours, allergic disorders and asthma2. Our results revealed that the most fern species have potential in selective COX-1 inhibitory activity. Significant inhibition to COX-1 was measured in *Dryopteris cambrensis* (92,46%) or *Athyrium distentifolium* (91,18%). Only a few fern species revealed moderate inhibition to COX-2 (*Dryopteris expansa* and *Dryopteris aemula*). Significant 5-LOX inhibitory activity was measured in *Onoclea sensibilis* (71,06%) and *Dryopteris caucasica* (68,32%). Our results reveal several European ferns, as a potential source of anti-inflammatory compounds.

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PXFL31

DETERMINATION OF TOXICITY OF BRAF INHIBITORS *IN VITRO*

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Malignant melanoma belongs between one of the most serious skin diseases nowadays. However, treatment of advanced melanoma is difficult and ineffective. A significant part of melanomas exhibits a mutation of the gen for oncogenic BRAF kinase, which is responsible for stimulation of proliferation and surviving of cancer cells. These melanomas can be treated using novel therapeutic approach encompassing specific BRAF inhibitors, dabrafenib or vemurafenib (1). Available clinical studies indicate that the toxic effect of the BRAF inhibitors is focused primarily on podocytes localized in the glomerular membrane. Our study is focused on evaluation of cytotoxic effect of BRAF inhibitors on selected model renal cells in vitro to confirm the renal target of toxicity. Main aim was to analyze whether renal toxic effect of BRAF inhibitors is specifically limited only to the podocytes or other renal cells may be damaged. The experiments were performed using human cell lines representing different types of kidney cells (HEK-293, PODO/TERT256, HK-2) and standard liver cell line HepG2 as a comparator. Amphotericin B and paracetamol were employed as comparative toxins. The IC_{50} values determined by analysis of inhibition curves were used for comparison. The found experimental data showed comparable toxic effect of the tested BRAF inhibitors in each used kidney cell line. However, vemurafenib exhibited significantly higher toxicity compared to dabrafenib. The in vitro toxicity of vemurafenib in all renal cell lines was even stronger than that of known renal toxin amphotericin B. The results may suggest that the toxic damage of kidney caused by BRAF inhibitors treatment encompass not only podocytes but also other kinds of kidney cells including renal tubular cells may be involved.

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PXFL32

THE EFFECTS OF THE CIGARETTE SMOKE ON HUMAN SKIN AND POSSIBILITIES OF ITS PROTECTION

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Cigarette smoke (CS) represents a significant source of air pollution with negative effects on human health. CS is strongly associated with the number of pulmonary and cardiovascular diseases, but it also induces significant damage of the skin which is often neglected. In the skin, one of the negative impacts of the long-term exposure to CS is the skin barrier impairment, premature skin aging and development of skin diseases such as atopic dermatitis, psoriasis, acne, even skin cancer.

In this study we investigated the effect of CS on the skin using porcine cadaver auricular skin explants that are very similar to human skin. After application of the CS on the surface of the skin samples, we observed significant negative changes in both the epidermis and dermis such as the presence of large amount of reactive oxygen species (ROS), oxidation of proteins and lipids, DNA damage, inhibition of collagen gene expression, upregulation of matrix metalloproteinase 1 and filaggrin and overall decrease in the skin cell viability.

Furthermore, this model was used to evaluate the ability of the selected compounds commonly used in cosmetics and dermatology with various mechanisms of action to prevent CS-induced skin damage. We tested several polysaccharides and their derivatives such as sodium hyaluronate (HA) of various molecular weight (MW: 1,7 MDa, 500 kDa, 15 kDa, 5 kDa), carboxymethyl-glucan, schizophyllan, glucomannan; then sodium ascorbyl phosphate (vitamin C), niacinamide (vitamin B3) and D-panthenol (provitamin B5). After incubation of the skin explants with the tested compounds and subsequent exposure to CS, the level of ROS and peroxidation of the skin barrier lipids together with gene expression of the proinflammatory cytokine IL-6 were determined. All the tested substances significantly protected the skin against CS from which the most effective was vitamin C due to its strong antioxidant properties and 1,7 MDa HA with its ability to create a protective film on the skin surface. The film-forming properties of HA were MW-dependent and correlated with its protective effect which both decreased with lower MW.

The results show that the exposure of the skin to CS leads to the significant skin damage which can be effectively prevented using some conventional cosmetic and dermatological ingredients with various mechanisms of action.

In the second part of our research we investigated the differences between the epidermis of smokers and non-smokers. For this experiment we used samples of the epidermis obtained by suction-blister technique from volar forearm of smoking (history of smoking min 15 years, min 15 cigarettes/day) and non-smoking women. We performed cDNA microarray analysis for the determination of the gene expression profile of the epidermis of

smokers and non-smokers. Significant differences were observed in the expression level of the number of genes with various functions. In the epidermis of smokers, we found significant upregulation of genes involved in the epidermal differentiation complex which was also confirmed using qRT-PCR and on the protein level.

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