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Abstracts

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BPT1

PCR ANALYSIS OF SELECTED MARKERS OF VASCULAR DAMAGE IN VASCULAR-TARGETED PHOTODYNAMIC THERAPY

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In vascular-targeted photodynamic therapy (VTP), photosensitizer (PS) is activated while it is still in the lumen of the vessels. Due to the short lifetime of singlet oxygen, the damage is limited to endothelial cells and vessel wall (1). The aim of this study was to evaluate the possible effect of four candidate compounds in VTP *in vitro* protocol on endothelial cells. Human Umbilical Vein Endothelial Cells (HUVECs) were treated with four different candidate phthalocyanine-based compounds (each representing one group – cationic symmetrical hydrophylic PS, cationic unsymmetrical amphiphilic PS, anionic symmetrical hydrophylic PS and anionic unsymmetrical amphiphilic PS) in concentrations corresponding to their EC_{15} and EC_{85}. Gene expression of selected markers that characterize endothelial cells was examined by quantitative real-time RT-PCR 3h and 12h after the treatment. PCR analysis demonstrated significant differences in the state of endothelial function in all four candidate compounds comparing to control. We observed similar changes in the mRNA level of markers of oxidative stress (HO-1), inflammation (PTGS2), and angiogenesis/endothelial damage (ENG and KLF6) using all PSs. In the remaining 12 markers, inter-individual differences (e.g. in mRNA level of VCAM or VEGF) were observed. In conclusion, these compounds directly affect chosen markers of endothelial function/dysfunction in HUVEC cells, however further studies (protein analysis) are needed to evaluate these effects.

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DRUG-INDUCED CARDIAC TOXICITY AND SENESCENCE

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Drugs can affect the entire cardiovascular system in a variety of ways, from direct cardiomyocytes' damage to impairment of cardiac development and repair. When cells are exposed to toxic non-lethal insult(s), cells can enter a specific state called stress-induced premature senescence leading to permanent cell-cycle arrest, structural and functional changes, esp. acquiring of the secretory phenotype. This phenomenon was found to be important in intercellular communications, propagation of the damage and its subsequent repair, and in developmental processes. Here we focused on effects of cobalt and bisphenols, and their link to stress-induced senescence as oxidative stress, hypoxia or epigenetic changes are among known triggers of stress-induced premature senescence. Cobalt cardiomyopathy is rare but severe consequence of cobalt intoxication (e.g., after metal hip arthroplasty) with unknown pathogenetic mechanism(s), except for the fact cobalt is well-known to induce hypoxia, and in high concentration oxidative damage. Bisphenol A and its derivatives (called "NextGen") are important in manufacturing of various plastics. Hence, they are found ubiquitously, but they also act as endocrine disruptors. For this reason, they are intensively studied for their possible harmful effects on humans including the heart, but there is little conclusive evidence. We observed some toxic effects of bisphenols on cardiovascular system in vitro and ex vivo only in high doses, therefore we focused on possible negative influence on differentiation of cardiac cells that was already described for bisphenol A. For this purpose, we are currently validating a model of differentiation of H9c2 cardiomyoblast cells into cardiac of striated muscle phenotype by different protocols.

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BPT 3

AFFIBLOT AS A TOOL FOR ANTIBODY QUALITY ASSESSMENT

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Immunoassay techniques use antibodies for the specific detection of proteins and finding an antibody with good specificity and selectivity is crucial for the quality of the data. The affiblot is a palm-size 3D printed dot-blot-based device that allows simultaneous semi-quantitative comparison of up to five antibodies regarding their affinity/avidity, cross-reactivity, and batch-to-batch reliability. During our research, we found that some commercial anti-Endoglin (Eng) antibodies show contradictory results regarding Eng expression during the development of atherosclerosis and non-alcoholic steatohepatitis (NASH). Thus, we aimed to assess the quality of the antibodies by evaluating the affinity and specificity of these antibodies and suggest the most suitable antibody for Eng detection in the aorta and liver in these pathologies.

For that, four different anti-Eng antibodies were evaluated for their affinity and specificity to mouse Eng. Affiblot (affinity and specific reaction with recombinant Eng) and western blot (WB) (cross-reactivity with non-target proteins) were used. The antibodies were tested with pure recombinant Eng and with liver and aorta homogenates either from healthy mice or from mice with induced atherosclerosis or NASH.

Results of this study showed that antibodies detected different number of bands by WB and some even failed to detect the target protein. Moreover, the antibodies displayed significantly different affinity to the target where some antibodies did not detect the differences in Eng expression which resulted in false negative biological results.

This device showed that only one of the tested antibodies has suitable parameters regarding specificity, sensitivity, and cross-reactivity for the identification of Eng reflecting biological differences in control and atherosclerotic/NASH samples. Thus, this study reflects the need to improve methods for the proper characterization of antibodies prior to the analysis of samples.

The study was supported by GACR 22-14961S and Charles University support program Cooperatio PharmSci.

BPT 4

EFFECT OF INFLAMMATION ON PLACENTAL TRYPTOPHAN METABOLISM: FROM CLINICAL STUDIES TO BASIC RESEARCH

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Intrauterine inflammation occurs in approximately 20% of all pregnancies and an alarming 85% of pregnancies leading to premature births (before 37 week of gestation). Several lines of evidence, including epidemiological data and results of animal research, clearly indicate links between maternal inflammation during pregnancy and increased risks of neurodevelopmental and psychiatric disorders in offspring. Among the mechanistic pathways through which maternal inflammation during pregnancy can affect fetal brain development and programming, the role of tryptophan (TRP) metabolism has received considerable attention, since various TRP metabolites possess neuroactive properties. In this study, we hypothesize that the hostile inflammatory environment may impair TPR metabolism in the placenta. To evaluate our hypothesis, we 1) examined gene expression signature of placental TRP metabolism and conducted metabolomic analysis in term and preterm placentas and 2) investigated TRP metabolism in term healthy placental explants exposed to inflammation. Our results show that several enzymes in TRP metabolism are differentially affected by inflammation, leading to impairment of the TRP pathway, particularly in the kynurenine (KYN) pathway. This dysregulation was characterized by reduced kynurenic acid (KYNA) levels and increased quinolinic acid (QUIN) levels, resulting in a shift towards excitotoxicity in the placenta exposed to inflammation. Collectively, the impairment of the placental KYN pathway during maternal inflammation exposes the fetus to significantly higher amount of potentially neurotoxic substances (QUIN).

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BPT 5

EXPLORING THE ANTI-INFLAMMATORY POTENTIAL OF PSYCHOTROPIC AND NON-PSYCHOTROPIC CANNABINOIDS IN HUMAN PLACENTA

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Cannabis abuse among pregnant women is a growing concern, with the increasing use of various cannabinoids, including tetrahydrocannabinol, hexahydrocannabinol, cannabidiol, cannabigerol, and cannabinol. Prenatal cannabis exposure has been linked to cognitive, attention, and memory deficits in fetal neurodevelopment. The mechanisms behind these effects are not fully understood, but the placenta - an organ crucial for fetal development, immune protection, and regulating inflammatory cytokines - may be involved. Pregnancy also poses a risk of inflammation, whether from infections or other sources, which can impair placental function and increase the risk of neurodevelopmental disorders in the child. Despite evidence linking placental dysfunction and abnormal fetal neurodevelopment, much about this relationship remains unknown. The objective of this study was to evaluate the pharmacological effects of exocannabinoids on specific inflammation-related molecules using an *ex vivo* model of healthy human placental explants exposed to an inflammatory stimulus. Healthy human placental explants were exposed to exocannabinoids for 48 hours, followed by a 4-hour treatment with lipopolysaccharide (LPS), a principal component of Gram-negative bacteria cell walls that elicits an acute inflammatory response. Following the treatment, the gene and protein expression, as well as the cytokine levels of IL1-β, IL-6, IL-18, TNFα, NLRP3, and Caspase-1 were analyzed. We observed that both psychoactive and non-psychoactive cannabinoids prevent the increase in LPS-stimulated inflammatory response. Our study raises the possibility that exocannabinoids may have immunomodulatory effects, a finding that is sure to spark controversy and further investigation.

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BPT 6

ENDOCANNABINOID SYSTEM IN PLACENTA: GENE EXPRESSION AND METABOLOMIC ANALYSIS IN HEALTHY AND PRETERM PREGNANCIES

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The endocannabinoid system (ECS) is a complex network of receptors and chemical signaling molecules that are found throughout the human body. It regulates multiple physiological processes, including pregnancy and childbirth. However, its role in gestation remains obscure. It has been reported that a placenta expresses ECS similar to the brain, and abnormalities in placental homeostasis may contribute to the onset of preterm birth – a leading cause of perinatal mortality and long-term morbidity. The objective of this study was to investigate the connection between changes in the placental ECS profile and preterm birth. Using gene expression and metabolomic analysis, we compared ECS component levels in preterm and term placenta tissues. We observed alterations in gene expression in preterm birth placenta of the main ECS enzymes: fatty acid amide hydrolase (FAAH), N-acylphosphatidylethanolamine-specific phospholipase D (NAPE-PLD), diacylglycerol lipase (DAGL), monoacylglycerol lipase (MAGL), alpha/beta-hydrolase domain containing 6 (ABHD6), and the following receptors: peroxisome proliferator-activated receptor gamma (PPARG), transient receptor potential cation channel subfamily V member 1 (TRPV1). The metabolomic data indicate that the ECS profile in preterm birth placentas is enormously different from those in term deliveries and that preterm birth is usually accompanied by higher levels of endocannabinoids. These findings suggest that the ECS may play a role in the development of preterm birth and warrants further investigation.

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BPT 7

MONOAMINE TRANSPORT IN THE TROPHOBLAST; EFFECT OF TROPHOBLAST DIFFERENTIATION AND DRUGS COMMONLY USED IN PREGNANCY

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In pregnancy, fetal development and programming rely strongly on the placental transport of essential primary monoamines (serotonin, noradrenaline, and dopamine). Transporters and enzymes serving the purpose can be found in the layer of trophoblast cells: syncytiotrophoblasts (STBs) and their precursors, cytotrophoblasts (CTBs). Polarization of STBs creates a maternal-side facing microvillous membrane (MVM), expressing the serotonin transporter (SERT) and noradrenaline transporter (NET), and a fetal-side facing basal membrane (BM), expressing organic cation transporter 3 (OCT3). Transporter expression variabilities upon differentiation have already been examined and our recent study supplemented the knowledge of their functional differences. Furthermore, studies suggest these transporters might be sensitive to various inhibitors commonly prescribed in pregnancy, such as antidepressants or antidiabetics (metformin), altering their function. Experimental approaches used included *in situ* and *in vitro* methods: rat term placenta perfusions and freshly isolated primary human trophoblast cells. In trophoblast differentiation studies, *in vitro* experiments were followed by gene/protein expression and functional analysis. BeWo cells have been included in some of the experiments to determine the appropriateness of their use in placental physiology research. Finally, our study describes how trophoblast differentiation, choice of the placental experimental model, and presence of drugs commonly used in pregnancy affect the monoamine uptake in the trophoblast.

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BPT 8

ANTIDEPRESSANTS IN PREGNANCY; ACUTE AND CHRONIC EFFECTS

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Depression is diagnosed in up to 25 % of pregnant women. Of these, around 10 % take antidepressant pharmacotherapy. In recent decades, antidepressants taken during pregnancy have been associated with a range of poor outcomes. Nevertheless, the underlying mechanisms are still unknown. In the brain, the effect of antidepressants on monoamine homeostasis is described in detail. In contrast, the same topic has never been studied in the placenta, despite monoamines (serotonin, norepinephrine, and dopamine) being crucial neuromodulators for fetal development and programming. Therefore, their imbalance in the fetoplacental unit may lead to lasting changes in the brain structure and function, projecting into adulthood. In the present study, we revealed serotonin transporter, norepinephrine transporter, and organic cation transporter 3 as the main membrane proteins responsible for monoamine uptake from maternal and fetal circulations, respectively. Further, we confirmed a strong inhibitory effect of six clinically used antidepressants in pregnancy on placental monoamine transporter function. Finally, we performed long-term treatment of pregnant rats with paroxetine; in this study, we focused on its effect on uteroplacental and fetoplacental circulation and on the expression of genes involved in monoamine homeostasis in the placenta and fetal brain. A wide range of experimental methods was used in this study, specifically, *in situ* rat placenta perfusion, uptake studies with *ex vivo* isolated human placental membrane vesicles, *in vitro* techniques using MDCK-II and HRP-1 cell lines, methods of molecular biology (qPCR, Western blot) and unique Doppler ultrasound. Collectively, our findings indicate novel mechanisms whereby antidepressants affect fetoplacental monoamine homeostasis and increase vascular resistance in uterine and umbilical arteries, contributing to poor pregnancy outcomes.

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BPT 9

A STUDY ON PHARMACOKINETIC ASPECTS OF RIFAMPICIN AND ITS DERIVATIVES IN LIVER CELL MODELS

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Although pharmacokinetic aspects of antibiotic rifampicin (RIF) have been extensively examined, there is less information on an action of its derivatives (RIFs) including both its metabolites and degradation products. Those are 25-desacetylRIF and 3-formylrifamycin SV generated from RIF by arylacetamide deacetylase (AADAC) and hydrolysis, respectively. Other RIFs are RIF N-oxide as well as RIF quinone, a product of nonenzymatic autoxidation.

In the study, RIFs were tested regarding their affinity towards pregnane X receptor (PXR), a regulator of CYP3A4 expression. Besides, gene expression changes of AADAC as well as RIF-related transporters OATP1B1, OATP1B3, and MDR1 were interrogated in long-term experiments with 3D primary human hepatocytes (PHHs) to unveil mechanisms behind autoinduction of RIF elimination, which is clinically observed after a repeated dosing.

First, 25-desacetylRIF revealed the lowest affinity towards PXR in gene reporter assay (EC50 = 86.57 μM) compared to other RIFs, but it could surprisingly contribute to CYP3A4 induction in 3D PHHs at a relevant concentration of 10 μM. The PXR affinity of other RIFs could be arranged as follows: 3-formylrifamycin SV (EC50 = 0.87 μM) > RIF quinone > RIF > RIF N-oxide (EC50 = 2.71 μM). Those compounds equally induced CYP3A4 in 3D PHHs.

All RIFs showed a direct binding to PXR resulting in about 3-fold activation of gene reporter construct in two hybrid assays, whereas 25-desacetylRIF showed 1.8-fold induction.

Our data further give rise to hypothesis that upregulation of MDR1 expression may increase RIF hepatic elimination. Although, other determinants in RIF autoinduction may also participate, those are unlikely AADAC, OATP1B1, and OATP1B3.

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BPT 10

THE EFFECT OF PRENYLFLAVONOIDS ON GENE EXPRESSION IN HEALTHY AND STEATOTIC MURINE PRECISION-CUT LIVER SLICES

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Non-alcoholic fatty liver disease (NAFLD) is a serious pathological state ranging from reversible steatosis or steatohepatitis to a more severe forms such as cirrhosis. Unfortunately, approved pharmacotherapy is not yet available, and the treatment of NAFLD is still complicated.[1] Prenylflavonoids (PF) are promising molecules that ameliorated steatosis and inflammation in mice *in vitro*.[2] This study aimed to identify the effect of two PF, 6- prenylnaringenin (6-PN) and xanthohumol (XH) on the expression of genes involved in xenobiotic and lipid metabolism in healthy and steatotic murine precision-cut liver slices (PCLS). Steatosis was induced by a medium containing fatty acids, fructose, insulin, and citrate. PCLS were incubated in the steatotic medium in the presence or absence of XH or 6-PN for 24 and 36 hours. Fat accumulation was determined by measuring triglyceride content. In healthy PCLS, both PF dysregulated the expression of various xenobiotic and lipid-metabolizing enzymes and acted as significant inducers of CYP2B and CYP2C29. Interestingly, these effects could no longer be observed in steatotic PCLS. Nevertheless, 6-PN demonstarted a protective effect in fatty PCLS as it increased the expression of antioxidant enzymes (GSTA1/2, GPx7) as well as CPT1A, an enzyme facilitating beta-oxidation of fatty acids.

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BPT 11

CHANGES IN THE GENE EXPRESSION IN THE IN VITRO MODEL OF STEATOSIS

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Non-alcoholic fatty liver disease (NAFLD) is a spectrum of liver diseases varying in severity of injury that develop in the absence of excessive alcohol use. It is characterized as a metabolic, multifactorial, and chronic liver disease with increasing worldwide prevalence. NAFLD affects about a quarter of population worldwide and has become an epidemic in parallel with the epidemic of obesity. Intake of high-fat diet causes an excessive fat accumulation in the liver referred to as simple steatosis, which can further progress to more severe stages of NAFLD including non-alcoholic steatohepatitis (NASH), fibrosis, cirrhosis, hepatocellular carcinoma, and eventually liver failure. As NAFLD has been reported to cause changes in liver homeostasis and metabolism of xenobiotics, the aim of this study was to investigate NAFLD-induced changes in the mRNA expression of selected liver enzymes in an in vitro model of NAFLD. For this purpose, murine precision-cut liver slices were incubated for 24 and 36 hours in a lipogenic medium containing a combination of fatty acids, fructose, citrate, and insulin to induce steatosis. Triglyceride content, a hallmark of NAFLD, was determined using colorimetric assay. The mRNA expression of selected genes encoding antioxidant and drug-metabolizing enzymes, and genes involved in lipid metabolism was determined using real-time quantitative PCR method. NAFLD significantly decreased the mRNA expression of several cytochrome P450 and glutathione peroxidase enzymes. Also, significant changes in the mRNA expression of genes involved in lipid metabolism were observed.

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CHANGES IN miRNA EXPRESSION IN TWO MOUSE MODELS OF NAFLD

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Non-alcoholic fatty liver disease (NAFLD) is a common cause of liver disease worldwide which is characterized by the accumulation of triglycerides in hepatocytes in the absence of excessive alcohol consumption. Non-alcoholic steatohepatitis (NASH), a more severe form of NAFLD associated with hepatic steatosis, inflammation, and hepatocyte damage, can further lead to fibrosis and cirrhosis. There is an urgent need to understand the pathogenesis of NAFLD and identify effective therapeutic targets.[1] MicroRNAs (miRNA) may play a crucial role in the emergence and progression of NAFLD. The aim of the present study was to compare the expression of selected miRNAs between two animal models of NAFLD. In the MSG model, newborn NMRI mice were injected subcutaneously with monosodium glutamate (MSG) to induce lesions in hypothalamic arcuate nucleus. MSG mice as well as age-matched wild-type NMRI mice (dietary model) were fed standard (STD) or high-fat, high fructose, high-cholesterol (FFC) diet for 5 months starting from the age of 2 months. Real-time quantitative polymerase chain reaction (RT-qPCR) was used to study miRNA expression. In the dietary model, the expression of miR-200b-3p was significantly increased by FFC diet compared to STD diet. In MSG model, the expression of miR-92a-3p and miR-122-5p were markedly reduced in mice fed with FFC diet compared to those on STD diet. Induction of NAFLD by MSG caused significant induction of miR-122-5p expression compared to wild-type mice (both on STD diet).

The study was supported by the Charles University Grant Agency (Project GAUK No. 225009).

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THE INTERACTION OF 6-PRENYLNARINGENIN WITH ABCB1

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Hops (*Humulus lupulus* L.) is a popular ingredient in various orally taken nutritional supplements to promote sleep or relieve postmenopausal problems. It contains prenylated flavonoids, such as 6-prenylnaringenin (6-PN), which are investigated for their potential anticancer and hepatoprotective activities. Therefore, in this study, we examined the absorption of 6-PN and its interaction with ABCB1, a known drug efflux transporter found in barrier systems limiting the absorption and distribution of its substrates. ABCB1 has multiple binding sites (for rhodamine123, digoxin, and Hoechst33342) and represents a site of clinically relevant drug-drug interactions. Using *in vitro* bi-directional transport studies across Caco-2 cells, we observed a significant increase in the efflux ratio of ABCB1 substrates rhodamine123 (1 μM) and digoxin (1 μM) caused by 6-PN (10 μM). We hypothesize that 6-PN binds to ABCB1 and induces conformation changes that support the transport of a substrate bounded on another site. This phenomenon was previously described with different substrates.[1] Another explanation could be the inhibition of uptake transporters on the apical membrane like OATP2B1, OCTN1, and OCTN2. However, in the follow-up *ex vivo* studies using human precision-cut intestinal slices, 6-PN (10 μM and 50 μM) did not significantly change the accumulation of either substrate. Experiments addressing 6-PN intestinal absorption and the role of ABCB1 in this process are currently being analyzed.

The study was supported by the project "Grant Schemes at CU" (reg. no. CZ.02.2.69/0.0/0.0/19_073/0016935).

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GENETICALLY MODIFIED CELL LINES USED IN NEW LRP2 LIGANDS DETERMINATION

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Low-Density Lipoprotein Receptor-Related Protein 2 (LRP2, megalin) is a member of LDL receptor family localized on the apical surface of many different epithelial cells. LRP2 is responsible for internalization of high molecular weight compounds.[1] Among receptor-mediated reabsorbed substances belong various proteins, nutrients and hormones.[2]

Human cell lines originally expressing LRP2 – HK2 (proximal tubular cell line) and JEG-3 (human placental choriocarcinoma cell line) were genetically modified using CRISPR/Cas9 technique. Designed sgRNA sequences target crucial motifs to harm LRP2 function. The gene editing was verified by viability and accumulation studies using well-known LRP2 ligands, aminoglycoside antibiotic gentamicin and FITC-albumin. The FACS (Fluorescence-Activated Cell Sorting) method was involved to separate modified cells from unmodified cells. After confirmation of lower LRP2 expression in sorted cells by qRT-PCR and verification of harmed function by control methods, modified cells can be further applied in potential LRP2 ligands testing. Radiolabeled VEGF-A N-terminal helix-derived 15 amino acid peptide with binding and inhibitory potency to VEGF (Vascular Endothelial Growth Factor) receptors was tested as potential megalin substrate. The accumulation assay confirmed the 15-mer as LRP2 ligand.

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ARE MULTICELLULAR SPHEROIDS A SUITABLE TOOL FOR PHOTODYNAMIC THERAPY RESEARCH?

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Photodynamic therapy (PDT) is approved non-invasive treatment method for cancer and also for several non-malignant diseases. PDT utilizes drugs called photosensitizers (PS), light with appropriate wavelenght and molecular oxygen. Commonly used two-dimensional (2D) cell lines are cost-effectineve and easy to use. However, cells growing in the monolayer do not provide the real complexity and 3D structure found in the human body. Spheroids should help better mimic tumour microenvironment, complex cell-cell and cell-matrix interactions.[1] In this work, we study photodynamic therapy of water-soluble (aza)phthalocyanine photosensitizers on 3D multicellular spheroid culture models (further reffered as "spheroids"). Spheroids were generated from HeLa (human cervical carcinoma), MCF-7 (human brest adenocarcinoma) or CT-26 (mouse colorectal carcinoma) malignant cell lines using ultra-low adhesion plates. Cytotoxicity of original amphipilic and hydrophilic (aza)phthalocyanines (cationic and anionic) was determined by two separate methods – luminiscence measurement of ATP and fluorescence measurement of resorufin formation. Distribution of PSs within spheroid strucutre was determined by confocal laser scanning microscopy. We were also interested in the invasive behavior of spheroids before and after photodynamic treatment with studied compounds. The amphiphilic cationic PS provided higher phototoxicity against all cell lines. Spheroids derived from CT-26 cell line were the only ones to show invasive behavior to extracellular matrix. All studied PSs were able to reduce invasivity after irradiation, nevertheless, cationic PSs were more efficient.

The study was supported by Charles University (SVV 260 550), by Czech Science Foundation (19-14758Y).

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CHARACTERIZATION OF DUAL-SPECIES BIOFILMS FORMED UNDER DIFFERENT CONDITIONS *IN VITRO*

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Microorganisms, including multidrug-resistant ones, prefer a lifestyle in complex communities called biofilms, which are mostly polymicrobial. These communities are more resistant to host defense mechanisms or antimicrobials.[1] One of the factors behind the enhancement of antimicrobial resistance in biofilms is (except the cell-to-cell communication) the presence of the biofilm matrix.[2] Reflecting this we are focused on *in vitro* formation of dual-species microbial biofilms from clinically relevant bacteria and fungi: methicillin-resistant *Staphylococcus aureus, Staphylococcus epidermidis*, and *Candida albicans* under different conditions and evaluations of their attributes such as viability, quantitative representation of individual species using fluorescent microscopy, total biofilm biomass using Crystal violet staining, the resistance after the exposure to 50-fold higher MIC of ciprofloxacin and anidulafungin using Alamar blue method, or quantification of the key attributes of biofilm matrix - proteins, saccharides, eDNA. The key impact of available nutrients in different cultivation media on the formation and character of biofilms has been revealed. After the finalization of all experiments, the linkage of biofilm matrix composition to increasing antimicrobial resistance will be revealed. Knowledge from this study can help with the clarification of the molecular mechanisms behind the resistance of biofilm-forming agents and subsequently with the rational therapy of polymicrobial biofilm infections.

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COMPARISON OF COAGULATION BETWEEN DIABETES MELLITUS TYPE I PATIENTS AND HEALTHY VOLUNTEERS: PRELIMINARY DATA.

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Diabetes mellitus type 1 (DMT1) is a disease characterized by the inability of the patient to produce sufficient insulin[1]. It is known that patients with DMT1 present a higher risk of cardiovascular events; particularly a higher incidence of thrombus formation is comprehended among the most serious [2,3.] In clinical practice, several laboratory tests can be used to evaluate the level of coagulability of a sample such as prothrombin time (PT) and activated partial thromboplastin time (aPTT), among others. In this project, plasma samples of healthy volunteers and patients with DMT1 were analyzed for differences in coagulation. For this purpose, DMSO (0.1%) samples, as well as samples pre-incubated with four anticoagulants (rivaroxaban (RIV), dabigatran (DAB), argatroban (ARG), and apixaban (API)) all in a final concentration of 1 μM, were tested. Positive control samples with heparin were included as well. Samples were collected at the University Hospital of Hradec Králové, and a total of 51 healthy volunteers and 43 diabetic patients were enrolled. Untreated (DMSO) samples and those spiked with RIV, DAB, and API from DMT1 patients had higher PT (INR values) than corresponding samples of matched healthy volunteers. Regarding aPTT results, significantly higher values were observed again in plasma samples from DMT1 patients spiked with DAB and heparin. These preliminary data suggest some differences between coagulation parameters from both groups.

The study was supported by the Czech Health Research Council (project No. NU21J-02-00021)

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EFFECT OF FLAVONOIDS ON ZINC AND ZINC-CONTAINING DEHYDROGENASES

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Flavonoids are a group of secondary plant metabolites that are commonly present in diet. Due to their structural properties, some flavonoids exhibit substantial chelating activity and can therefore interfere with the homeostasis of trace metals.[1] This disruption of essential metal homeostasis is common in chelation therapy used for metal overload, due to the lack of selectivity of currently available chelators. I have focused on zinc, as it is crucial for the activity of a plethora of enzymes and transcription factors.[2]

Following an initial screening of chelating properties of 21 various flavonoids, the most active chelators were further evaluated. The effect of 9 flavonoids on yeast and human zinc-containing alcohol dehydrogenases as well as on glutamate dehydrogenase, which is inhibited by zinc ions, was examined *in vitro*. The results show that chelation is not the main mechanism of inhibition of zinc-containing enzymes. For the most efficient inhibitors, docking studies were also performed and have confirmed this initial assumption. Taxifolin was a potent yeast alcohol dehydrogenase inhibitor but had no effect on the remaining enzymes. Baicalein had an effect on both alcohol dehydrogenases and also possessed some chelating properties which can explain its effect on zinc-mediated glutamate dehydrogenase inhibition. Luteolin was the most potent inhibitor overall while having negligible chelating activity. In conclusion, some flavonoids can significantly affect the activity of zinc-containing enzymes, and this may have some biological consequences, but this inhibition is unrelated to their chelating properties.

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BPT 19

PLATELET RESPONSIVENESS IN FAMILIAL HYPERCHOLESTEROLEMIC PATIENTS TREATED WITH PROPROTEIN CONVERTASE SUBTILISIN/KEXIN TYPE 9 MONOCLONAL ANTIBODIES

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Platelet hyperfunction and high low-density lipoprotein cholesterol (LDL-C) level are main risk factors for coronary artery disease (CAD). Patients suffering from familial hypercholesterolemia (FH) has multiple time higher LDL-C and they die prematurely of CAD even at a young age[1-2]. 15 patients treated in University Hospital Hradec Králové were enrolled in this study. 12 of 15 were taking the novel PCSK9ab therapy and 8 of 15 were also undergoing LDL-apheresis. Blood samples from all patients including pre- and post-apheresis period were tested for platelet aggregation induced by various 7 inducers as well as the effect of 3 clinically used drugs (acetylsalicylic acid, ticagrelor and vorapaxar) was analysed. PCSK9ab treated patients had comparable platelet aggregability to samples after apheresis with exception of ristocetin induced aggregation. TRAP induced aggregation in PCSK9ab patients was even less expressed in apheresis patients. Treatment of FH patients significantly decreased platelet aggregation in case of 5 from 7 inducers and improved the effect of 2 from 3 used drugs compared to age-matched healthy volunteer group. This study showed the suitability of PCKS9ab treatment in normalizing platelet function in FH patients.

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BPT 20

VOLTAGE-GATED POTASSIUM (K_V) CHANNELS CONTRIBUTE TO 3-METHOXYCATHECOL-INDUCED VASORELAXATION ON RAT AORTA *EX VIVO*

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Substituted catechols detected in human biological fluids can originate from the environment, meal (e.g., flavourings and smoke-cured products) or also arise upon flavonoid metabolism by the gut microbiota. One of these metabolites, 4-methylcatechol, exerts *ex vivo* vasorelaxant and *in vivo* blood pressure-decreasing effects in rats. This study aimed to screen its 22 closely-related derivatives for their *ex vivo* vasodilatory activities on the isolated rat aorta. Thirteen compounds produced almost complete vasodilation which was concentration-dependent. 3-methoxycatechol (3-MOC) was the most potent one with an EC_{50} value of 9.6 μmol.L^{-1}. Mechanistic experiments showed that the vasodilatory effects of sodium nitroprusside (NO donor) or forskolin (adenylate cyclase activator) were potentiated by 3-MOC. Subsequent testing showed that 3-MOC targeted directly the vascular smooth muscle probably *via* K_V7 channels as shown in two different models using both non-selective (4-aminopyridine) and selective (linopirdine) K^+ channel inhibitors. On the other hand, direct effects on vascular endothelium, to muscular BK_{Ca}, K_{ATP}, K_{IR} or L-type Ca^{2+} channels or to muscular sGC, PKG or SERCA can be excluded. In short, these findings suggest that 3-MOC-induced vasodilation *ex vivo* is based on activation of K_V channels, direct or indirect, with re/hyperpolarization of the vascular smooth muscle.

The study was supported by the Grant Agency of Charles University (136120/C).

BPT 21

DIFFERENCES IN PLATELET-MONOCYTE AGGREGATES OCCURRENCE BETWEEN HEALTHY VOLUNTEERS AND FAMILIAL HYPERCHOLESTEROLEMIA PATIENTS

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Platelet-monocyte aggregates (PMA) have been proposed as a modern biomarker of pathologic platelet activation in some cardiovascular diseases such as coronary artery disease, including unstable angina and acute myocardial infarction[1]. Theoretically, a higher number of aggregates formed in individuals with abnormal lipid profiles are expected since platelets can be activated by oxidized low-density lipoprotein[2]. The aim of this study was to compare by using flow cytometry the amount of PMA in blood samples of healthy volunteers and patients with familial hypercholesterolemia (FH), a quite rare condition in which cholesterol levels are increased due to impaired lipid metabolism, mainly of low-density lipoproteins (LDL)[3]. In this study, we enrolled a total of 50 healthy volunteers and 15 FH patients (8 undergoing apheresis, 7 only treated pharmacologically). To study the effect of clinically used anticoagulants drugs, plasma samples were pre-treated with rivaroxaban, apixaban, dabigatran, or argatroban at a final concentration of 1 μM and the PMA number was compared to control samples. Our results show that PMA incidence was significantly higher in FH patients compared to the healthy donors. In addition, differences were also observed in the FH patient group depending on the treatment strategy (physical or pharmacological). Contrarily, the treatment of plasma samples with anticoagulants did not influence PMA formation in FH patients.

The study was supported by Czech Health Research Council (Grant No. NU21J-02-00021)

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WHAT IS THE ROLE OF ENDOGLIN AND SOLUBLE ENDOGLIN IN DIFFERENT LIVER PATHOLOGIES?

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Endoglin (CD105) exists in two forms, particularly membrane endoglin (Eng) and soluble endoglin (sEng) circulating in the blood. It has been established that Eng would possibly play an essential role in the process of liver impairment. However, the precise impact of changing in Eng expression, signaling, and sEng levels during these pathological changes in the liver are still unknown. The purpose of this study was to analyze the expression of Eng and sEng levels with respect to biomarkers of fibrosis, inflammation, and endothelial dysfunction in two mouse models of liver injury. The liver damage was induced in 9-12-weeks-old C57BL/6 male mice by DDC (3,5-diethoxycarbonyl-1,4-dihydrocollidine) and CDAA-HFD (choline-deficient L-amino acid defined high-fat diet) diets, while control animals were fed with a chow diet for a duration of four weeks. DDC diet was used to mimic the development of intrahepatic cholestasis, and the CDAA-HFD diet induces NASH changes. The liver impairment was confirmed by the significant increase in the level of liver enzymes along with increased expression of fibrosis and inflammation markers in both mouse models of liver fibrosis. DDC and CDAA-HFD diets significantly increased sEng levels and MMP-14 liver expression. While a significant reduction in the liver expression of Eng was observed in the DDC animals, there was a significant upregulation of Eng protein expression in the CDAA-HFD group. We suggest that both cholestasis and NASH induction result in cleavage of Eng by MMP-14, presenting sEng as a biomarker of these pathologies. Additionally, decreased Eng expression in DDC mice might be related to the development of liver sinusoidal endothelial dysfunction without its role in liver fibrosis. On the other hand, increased Eng expression in the CDAA-HFD group might indicate its possible role in fibrosis development. Thus, we propose the different potential role of endoglin in various liver pathologies, which will be further explored in our lab.

The study was supported by GACR 22-14961S, GAUK 362221, and SVV/2021/260549.

POTENTIAL INVOLVEMENT OF ENDOGLIN IN CORONARY ARTERY ENDOTHELIAL DYSFUNCTION FROM DIABETIC PATIENTS – A PILOT STUDY

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Endoglin (Eng) is a major cell membrane glycoprotein expressed in the vascular endothelium, which is related to endothelial dysfunction and inflammation. Our previous experiments showed that stimulation of endothelial cells with high glucose resulted in increased levels of Eng and proinflammatory cell adhesion molecules (ICAM-1 and VCAM-1) (1). We hypothesized that similar activation of Eng, endothelial dysfunction markers and an increase in adhesion/transmigration could be detected in cells from diabetic patients. Thus, we aimed to evaluate the impact of type II diabetes mellitus on Eng expression and function with respect to endothelial dysfunction and inflammation, by comparing human coronary artery endothelial cells from healthy donors (HCAEC) and human coronary artery endothelial cells from type II diabetes mellitus patients (D-HCAEC). Western blotting and flow cytometry were used to determine proteins levels of Eng, VCAM-1, ICAM-1. For functional analysis, THP-1 monocytes adhering to endothelial cells were quantified by flow cytometry. Transmigration assay was performed using cell culture inserts and transmigrated cells in the lower compartment were counted. ELISA assay was used for the quantification of soluble endoglin (sEng) in culture media. The protein analysis showed that Eng, sEng, VCAM-1 and ICAM-1, as well as adhesion and transmigration of THP-1 monocytes to endothelial monolayer, are significantly increased in D-HCAEC compared to HCAEC. These results imply that the presence of type II diabetes mellitus induces proinflammatory phenotype and increases both membrane and soluble Eng levels in endothelial cells, suggesting Eng involvement in type II diabetes mellitus, which will be further explored in prospective studies.

The study was supported by GAUK 288322 and SVV 260549.

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BPT 24

BRUTON´S TYROSINE KINASE INHIBITOR TIRABRUTINIB COUNTERACTS ANTHRACYCLINE RESISTANCE BY TARGETING ALDO-KETO REDUCTASE 1C3

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Bruton's tyrosine kinase (BTK), belonging to non-receptor tyrosine kinases of the TEC family, is one of the important targets of anticancer therapy. Consequently, it is a promising target for developing new small-molecule inhibitors. In clinical trials, BTK inhibitors were tested alone or in combination with anthracycline (ANT)-containing standard chemotherapy. ANTs represent the standard in oncological practice due to their high efficacy. However, their application is endangered by the emergence of resistance. Failure of ANT therapy may be caused due to several mechanisms among which the metabolic inactivation mediated through the activity of carbonyl-reducing enzymes (CREs) also belongs. AKR1C3, the most active enzyme from the superfamily of aldo-ketoreductases catalyses the two-electron reduction of ANTs to secondary alcohols generating metabolites. Our preliminary data indicate the ability of BTK inhibitors to inhibit ANT metabolism and improve the clinical outcome of ANTs. Tirabrutinib (TIR) is a small molecule of BTK inhibitor and was approved for the treatment of recurrent or refractory primary central nervous system lymphoma. Our project aims to describe the effect of TIR on daunorubicin reduction catalysed by AKR1C3. First, we performed the incubation methods with recombinant enzymes. Next, we continued with the inhibitory studies and drug combination assays in intact cells. The achieved results confirm the ability of TIR to inhibit the recombinant AKR1C3 and inhibit the AKR1C3 at the level of intact cells as well. Subsequent experiments proved a significant antiproliferative effect of the combination. In conclusion, TIR is an effective modulator of the AKR1C3 activity and the combination of TIR with daunorubicin might be an effective approach to ANT resistance.

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COBIMETINIB ANTAGONIZES ABCB1 AND CYP P450 ISOENZYMES MEDIATED CHEMOTHERAPEUTIC DRUG RESISTANCE

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Cobimetinib is a MEK inhibitor approved to be used in combination with vemurafenib for metastatic melanoma. In this study, we aimed to investigate if cobimetinib can cause pharmacokinetic drug interactions and could play a role in multi-drug resistance (MDR) in ATP binding cassettes (ABC) and CYP450 isoenzymes. In accumulation assays with fluorescence substrate, we could see cobimetinib showed potent inhibition towards the ABCB1 transporter while showing less potent inhibition towards the ABCG2 transporter. This was further confirmed using the cytotoxic substrates daunorubicin for ABCB1 and mitoxantrone for ABCG2, similar results were seen. Cobimetinib was also confirmed as an inhibitor of CYP3A4 and CYP2D6 enzymes. In combination studies cobimetinib synergistically reversed daunorubicin and mitoxantrone resistance in cells with ABCB1 and ABCG2. Furthermore, cobimetinib was also seen to antagonize CYP3A4 mediated resistance towards docetaxel. In our final experiment, which was the induction studies, we saw that cobimetinib did not increase the mRNA levels of ABCB1, ABCG2, and ABCC1. In summary, our findings show that ABCB1 affects cobimetinib transport across the membranes and the high potential of cobimetinib drug-drug interactions (DDIs) through ABCB1 and ABCG2 transporters and CYP isoforms.

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BPT 26

TALAZOPARIB EFFECTIVELY MODULATES PHARMACOKINETIC RESISTANCE MEDIATED BY ABCG2 AND ABCC1: AN *IN VITRO* AND *EX VIVO* STUDY

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Talazoparib (Talzenna) is a novel medication approved for the therapy of breast cancer. In this study, we explored its inhibitory interactions with ABC drug efflux transporters and described its chemosensitizing properties. *In vitro* accumulation studies showed that talazoparib inhibits ABCG2 and ABCC1 transporters, which was confirmed by molecular docking. Next, talazoparib synergistically reversed cytostatic resistance in cells with ABCG2 and ABCC1 overexpressions *in vitro*. Importantly, resistance-modulatory capabilities were subsequently verified in *ex vivo* patient-derived lung cancer explants with differential expressions of ABCG2 and ABCC1. We found clear association between the outcomes of expression, accumulation and drug combination assays. Explants with high transporters' expression and significant accumulation changes produced synergistic effects, whereas low-expressing ones with no accumulation differences yielded additive or antagonistic effects, respectively. In conclusion, our data introduced talazoparib as a potent antagonist of pharmacokinetic resistance. These findings might be translated into the effective and safe therapeutic strategy in future.

The study was supported by the Grant Agency of Charles University (project No. 334120/C), the Czech Science Foundation (grant No. 20-20414Y) and Charles University (SVV 260 549).

BPT 27

GILTERITINIB RESISTANCE IN ACUTE MYELOID LEUKEMIA

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Acute myeloid leukemia (AML) is a blood cancer, in which drug resistance is one of the most challenging obstacles. Even agents newly introduced in the therapy protocols end up being ineffective overtime due to rapid adaptability of cancer cells. In this study, we focused on mechanisms leading to gilteritinib resistance, a drug recently approved for the treatment of relapsed and refractory AML. We developed gilteritinib-resistant HL-60 G75 cell line, which acquired transient resistance to the drug. Transcriptomics and proteomics analysis revealed distinct gene and protein profiles of HL-60 G75 when compared to gilteritinib-sensitive HL-60 WT cells. Although HL-60 G75 appeared to have developed resistance by a multifactorial adaptation to gilteritinib selective pressure, some processes seemed to be more deregulated than others including lysosomes-related processes. Lysosome-specific staining identified elevated number of lysosomes in HL-60 G75, which decreased immediately after gilteritinib withdrawal. Sequestering capacity of lysosomes was verified using sunitinib. In HL-60 G75, sunitinib accumulated in lysosomes, while in gilteritinib-depleted HL-60 G75, it was predominantly spread within the cytosol. We suppose that fluctuation of lysosomal mass is highly dependent on gilteritinib presence, and its increase might be a result of activated lysosomal biogenesis, yet the exact mechanism will have to be further investigated.

The study was supported by Charles University, Czech Republic (PRIMUS/20/MED/010, SVV/260-549 and GAUK/205019).

EXTRACELLULAR MATRIX-RELATED SIGNALING PARTICIPATES IN THE DEVELOPMENT OF RESISTANCE TO EGFR-TARGETING TYROSINE KINASE INHIBITORS

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The emergence of drug resistance has obscured the success of EGFR–tyrosine kinase inhibitors (EGFR-TKis) in clinical oncological practice. Extracellular matrix (ECM) is a key component of tumor microenvironment, which regulates various biological activities of tumors. The present study aimed to uncover the possible universal roles of ECM-related signaling in EGFR-TKis' resistance. First, we generated three cell lines resistant to gefitinib, dacomitinib and osimertinib. Established drug resistant cells exhibited higher invasiveness than the parental variants in 3D organoid culture. Next, results of global proteomic analysis revealed that the aberrant activation of ECM-associated signaling may cause resistance to EGFR-TKis. Western blotting confirmed that both FAK and YAP1 pathways were upregulated in all resistant cell lines. In drug resistant models, pharmacological inhibition of FAK and YAP1 synergically enhanced the anticancer activities of EGFR-TKis. Interestingly, suppression of FAK pathway attenuated the hyperinvasive behaviors of drug resistant cells. In conclusion, these results indicated that ECM-related signaling (FAK and YAP1) actively participates in establishing EGFR-TKis' resistance. Our results might serve as a theoretical support for the development of treatment strategies to combat this problematic phenomenon.

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BIOLOGICAL ACTIVITY OF EUROPEAN FERNS

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In our previous study, European ferns were exposed to several different biological assays. They were tested for anti-oxidative[1], anti-inflammatory, anti-cancer and anthelmintic activity. Given the increasing request for natural pharmacological molecules, crude extracts and fractions of selected fern species (*Dryopteris borreri*, *Dryopteris remota*, and *Onoclea sensibilis*) were tested for further biological activities. Its anti-bacterial effect and inhibition of elastase and tyrosinase enzymes were assessed at the Department of Plant and Soil Sciences at the University of Pretoria in South Africa under the leadership of Prof. Namrita Lall.

Based on obtained results, crude extracts and selected fractions of both Dryopteris species showed excellent activity[2] in inhibiting the Gram-positive bacterium *Cutibacterium acnes*. While crude extracts of *Onoclea sensibilis* and *Dryopteris remota* showed the best results in the inhibition of elastase (IC_{50} 15.64±1.22 μg ml^{-1} and 31.07±7.81 μg ml^{-1}) and tyrosinase (IC_{50} 35.8±13.48 μg ml^{-1} and 40.64±3.44 μg ml^{-1}) enzymes.

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EXPRESSION OF SELECTED GENES IN *HAEMONCHUS CONTORTUS* EXPOSED TO SUB-LETHAL DOSES OF FENBENDAZOLE

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The nematode *Haemonchus contortus*, a gastrointestinal parasite of ruminants, endangers the health of farm animals and has a significant impact on livestock production. Anthelmintics are the mainstay in the prophylaxis and treatment of haemonchosis, but their efficacy rapidly diminishes due to drug resistance development. An increased anthelmintics inactivation via biotransformation belongs to a significant drug-resistance mechanism. Nematodes, including *H. contortus*, possess a relatively large number of drug-metabolizing enzymes (DMEs) compared to other helminths. For this reason, three DME super-families attract particular attention: cytochromes P450 (CYPs), UDP-glycosyltransferases (UGTs), and ATP-binding cassette (ABC) transporters.

Previous study[1] showed a significant induction of several DME genes of *H. contortus* after exposure to sub-lethal doses of albendazole (ABZ), an anthelmintic from the benzimidazole family. The effect of sub-lethal concentrations of fenbendazole (FEN), another benzimidazole anthelmintic, on *H. contortus* adults was evaluated. Although our results did not confirm the induction effect of FEN as ABZ, we cannot rule out the possible induction of other DME genes. Subsequent evaluation of FEN biotransformation will guide our next steps.

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UHPLC/MS ANALYSIS OF FENBENDAZOLE METABOLITES IN *HAEMONCHUS CONTORTUS*

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Haemonchus contortus, which belongs to the group gastrointestinal parasitic nematode, is well known in most of the world because it causes significant economic losses to the livestock industry. In addition, resistance to all anthelmintic classes has been reported. The study of metabolic pathways of anthelmintics is substantial for the efficacy of therapy and evaluation of the risk of drug-resistance developments in nematodes[1]. Fenbendazole (FEN) is a broad-spectrum anthelmintic drug that is used to a large extent[2]. The aim was to conduct the biotransformation study of FEN, which was not done before. Three different strains of *H. contortus* were used: benzimidazole susceptible strain ISE (Inbreed susceptible Edinburgh), benzimidazole resistant strain IRE (Inbreed resistant Edinburgh) and multi-resistant strain WR (White river)[2]. Individual nematode and media samples were extracted by solid-phase extraction (Strata-X). A UHPLC system (Dionex Ultimate 3000) and an HRMS analyzer (Q Exactive Plus) were used to analyze the resulting structures. During the analysis, the anthelmintic FEN metabolites were identified, formed by hydroxylation, hydrolysis and glycosidation; their abundance varied depending on the *H. contortus* strain.

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CONSTRAINT-BASED METABOLIC MODEL OF THE PARASITIC NEMATODE *HAEMONCHUS CONTORTUS*

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Parasitic nematodes threaten the health of the human population and represent a major burden on livestock and crop production. Pharmacotherapy, the mainstay in combat against these parasites, has become less and less efficient due to development of drug-resistance in nematodes. Therefore, only detailed knowledge of the metabolism of each nematode species will make prevention and treatment possible in the future. Great advances in "omics" technologies, bioinformatics, and computational biology can be used to understand the metabolism of nematodes properly. We used a computational approach to create a constraint-based model (CBM) for *Haemonchus contortus* applying a new reconstruction pipeline. CBMs were developed shortly after the first microbial genomes were sequenced and they rely directly on the genomic information and biological databases in order to predict metabolic functions through gene-protein-reaction mechanisms. These models allow us to simulate the growth of *H. contortus in silico* as well as test various hypothesis and provide an alternative to traditional experimental approaches. The created metabolic model of *H. contortus* can be a building block for future research such as the identification of potential drug targets and mechanisms of drug-resistance.

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THE POTENTIAL OF FERNS EXTRACTS AGAINST HAEMONCHUS CONTORTUS

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Since nematode *Haemonchus contortus* has become one of the most problematic and widespread parasites of small ruminants around the world, a lot of effort has been made to find an effective treatment. On the market, several anthelmintic drugs are losing their effectiveness because of the drug-resistance development in nematodes. For this reason, new effective substances are extremely needed. Apart from synthesizing new chemical entities or drug repurposing, plants have been important in treating several diseases in humans and animals for centuries. Thus, in our project, we were exploring the anthelmintic efficacy of selected fern extracts against *H. contortus.* The potential hepatotoxicity of fern extract was also tested. From eight fern species studied, the two of them: *Athyrium distentifolium* and *Dryopteris cambrensis* showed to be promising and worthy of future testing.

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METABOLISM OF 6-PRENYLNARINGENIN *IN VITRO*

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Hop prenylflavonoids have been recently broadly investigated due to their numerous positive effects on human health. Exposure to these natural compounds occurs primarily through beer consumption.[1] Among these prenylated flavonoids, 6-prenylnaringenin (6-PN) demonstrated strong antifungal, antibacterial, and antiproliferative activity *in vitro*[2], as well as alleviated neuropathic and visceral pain *in vivo*.[3] Despite the promising health benefits of 6-PN, the information about its pharmacokinetic properties in human or other animal species is still limited. To address this issue, we investigated the hepatic and intestinal metabolism of 6-PN in human precision-cut tissue slices. In addition, the hepatic metabolism was also studied in murine and rat precision-cut liver slices. UHPLC-MS/MS analysis was employed for this purpose. 6-PN was metabolized into several phase I and phase II metabolites in all analyzed tissue slices. Among the identified metabolites, glucuronides and/or sulfates of 6-PN were the most abundant. Furthermore, several quantitative as well as qualitative interspecies differences in the metabolism of 6-PN were observed.

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MONITORING OF THE FENBENDAZOLE IN THE ENVIRONMENT

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Veterinary drugs are neglected, but hazardous environmental contaminants [1]. In particular, the use of veterinary drugs in large quantities in livestock therapy might represent high risks for ecosystems [2-4]. Drugs used in livestock farming mainly enter the environment directly via excrements on the pastures or via manure from treated animals used to fertilization of fields with fodder plants. Drugs and their metabolites reach soil or water [5] and might be absorbed by plants. Moreover, contact of helminths with traces of anthelmintics during their juvenile free-living stages (via contaminated soil and/or water) or during the parasitic stage (sheep's blood system) might promote the development of drug resistance in these parasites. Despite these facts, the presence and risks of anthelmintic drugs in the environment are understudied and underestimated.

This study aimed to assess the genuine environmental concentration of fenbendazole to estimate the extent of soil pollution in grazing. The QuEChERS sample preparation method was optimized for soil samples. LC-MS /MS method in SIM mode was applied as a powerful analytical tool.

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