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Abstracts

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BPT1

FLAVONOID METABOLITE 3-HYDROXYPHENYLACETIC ACID RELAXES PORCINE CORONARY ARTERY *EX VIVO*: A MECHANISTIC STUDY

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Regular intake of polyphenol-rich food has been associated with a wide variety of beneficial health effects, including the prevention of cardiovascular diseases. However, the parent flavonoids have mostly low bioavailability and, hence, their gut microbiota-derived metabolites have been hypothesized to be bioactive. One of these metabolites, 3-hydroxyphenylacetic acid (3-HPAA), was previously reported to produce vasodilation. The aim of this study was to elucidate the mechanism of this effect using the precontracted porcine coronary artery segments *ex vivo* and isometric tension recordings. The vasodilatory effects of 3-HPAA were partially dependent on presence of intact endothelium and functional endothelial nitric oxide synthase. In contrast, the direct participation of SKCa or IKCa channels, muscarinic receptors, cyclooxygenase nor L-type calcium channels was not observed. These findings suggest that the vasodilatory action of 3-HPAA might be at least partially based on the release of nitric oxide by the endothelial layer.

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BPT 2

COMPARISON OF THE EFFECTS OF CLINICALLY USED ANTICOAGULANTS IN A COHORT OF HEALTHY DONORS

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Anticoagulants are used for the prevention and treatment of thrombosis. Disproportionate effects are however, associated, with the opposite, the risk of bleeding[1]. Moreover, traditional anticoagulants have a number of limitations, including variable pharmacologic/ pharmacokinetic properties, and proneness to food and drug interactions, which can be overcome by newer type of anticoagulants such as direct thrombin inhibitors (DTI) and direct factor Xa (FXa) inhibitors[2,3]. The aim of this study was to compare the anticoagulant effect of novel direct anticoagulants (rivaroxaban, dabigatran, argatroban, and apixaban) and traditional heparin in terms of prothrombin time (PT) and activated partial thromboplastin time (aPTT) using plasma of a total of 50 healthy donors. The most active anticoagulant at the equimolar concentration (1 μM) in the PT test was rivaroxaban followed by apixaban and argatroban, while dabigatran was the least potent. For aPTT test, the activity order was the following: argatroban > dabigatran > rivaroxaban > apixaban. Correlation analysis showed interesting outcomes. For instances, the effect of dabigatran and argatroban was decreased with BMI values. On the other hand, no differences between men and women were detected. These results are a first step of an ongoing project in which we will compare these data to samples of patients suffering from different metabolic diseases.

The study was supported by Czech Health Research Council (NU21J-02-00021)

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SCREENING OF COBALT CHELATORS

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Cobalt is an essential trace element, but it can also rarely cause cobalt toxicity due to its release from cobalt-containing medical devices. Currently, there are no approved selective cobalt chelators which would represent an optimal treatment modality. Hence, the aim of this work was screening of potential cobalt chelators along with evaluating of their potential toxicity. Firstly, a simple spectrophotometric assay employing 1-nitroso-2-naphthol-3,6-disulfonic acid disodium salt (NNDSA) as an indicator was used for detection of cobalt chelation. In the second part, the potential toxicity of cobalt-chelator complexes was tested by HPLC method with coulometric detection, because cobalt can catalyse the Fenton reaction, which produces hydroxyl radicals ($OH^{\cdot}$). Also a more biological assay on isolated erythrocyte was employed where the degree of haemolysis was tested in both presence and absence of cobalt ions. Of the 20 synthetic chelators tested, 15 had a significant cobalt chelating effect. The chelating effect is dependent on the chelator-cobalt ratio and in some of them it was pH-dependent and more pronounced at neutral pH conditions than at acidic. The most active compounds which reached 100% chelation of cobalt ions in a ratio 10:1, chelator to cobalt (II), or lower, were EDTA, DTPA, nitroxoline, chloroxine, clioquinol and ADR-925. Some substances led to prooxidation and lysis of erythrocytes in the presence of cobalt.

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BPT 4

METABOLISM AND TOXICITY OF ANTHRACYCLINE ALCOHOL METABOLITE TOWARD NEONATAL RAT CARDIOMYOCYTES

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Daunorubicin (DAU), one of the anthracycline antibiotics (ANTs), is among the most potent anticancer drugs, but the potential limitation in their use is a risk of severe cardiotoxicity.[1] Some hypotheses have been proposed to elucidate this mechanism(s); e.g., the oxidative stress hypothesis, more recently the influence of topoisomerase IIβ, and the next hypothesis is related to the metabolism of ANTs in heart tissue. Nevertheless, the cardiotoxic potential of these metabolites is somehow inconsistent. Therefore, the influence of DAU and daunorubicinol (DAU-ol) on isolated neonatal rat ventricular cardiomyocytes (NVCM) was investigated.

At first, the pharmacokinetics study was performed. The NVCM were treated with DAU or DAU-ol and surprisingly after 24 hours the concentration of DAU-ol in cells was comparable. It means, that the same concentration of DAU-ol was enzymatically produced by NVCM and the same was allowed by intake to the cells. The toxicity profile was confirmed by two different methods (LDH and sytox); DAU-ol was less cardiotoxic compound, above that with significant protection of dexrazoxane (DEX). Moreover, DEX did not influence the pharmacokinetics of DAU or DAU-ol. In antiproliferative experiments on HL-60 leukemic cells showed DAU-ol partially maintained effect, with IC_{50} 4time higher than for DAU. Accordingly, obtained data show a lower cardiotoxic potential of DAU-ol toward NVCM with comparison to DAU, without the influence of DEX and maintained antiproliferative effect toward leukemic cells.

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BPT 5

REVIEWED EFFECTS OF XK469 ON TOPOISOMERASE 2 ACTIVITY AND ITS CONSEQUENCES FOR CARDIOPROTECTION

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Sodium-(2-[4-(7-chloro-2-quinoxalinyloxy)phenoxy]propionate (XK469) was derived from the herbicide Assure and has antiproliferative activity in solid tumors[1]. Although it proceeded to the second phase of several clinical trials, its mechanism of antineoplastic action is still not elusive. In the initial study by Gao et al. in 1999, XK469 is referred to act as selective TOP2B poison[2]. Furthermore, XK469 induced cell cycle arrest in the G2-M phase, activated apoptosis and increased TOP2A expression. As TOP2B inhibition is nowadays discussed as an important factor in the management of anthracycline cardiotoxicity [3], we study the effects of XK469 in this regard. In the presented study we aimed to further characterize the mechanisms of XK469 action, and due to its declared TOP2B selectivity, evaluate its cardioprotective potential. The kDNA decatenation assay for TOP2 activity revealed that XK469 inhibits both enzyme isoforms, although the IC_{50} for TOP2B was slightly higher than for TOP2A. In both rat neonatal cardiomyocytes (NVCM) and HL-60 cells, XK469 did not induce the accumulation of TOP2-DNA covalent complexes. Since the TOP2-DNA complexes eventually lead to DNA double-strand breaks, their formation was determined by immunodetection of H2AX phosphorylation and the Comet Assay. Additionally, cardiotoxicity/cardioprotection data was acquired in our well-established model in NVCM.

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BPT 6

POTENTIAL OF TOPOISOMERASE II INHIBITORS IN PREVENTION OF ANTHRACYCLINE CARDIOTOXICITY

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Anthracyclines (ANTs) retain indispensable role in anticancer therapy despite the risk of severe chronic cardiotoxicity. Only one agent was proven to be effective for its pharmacological prevention and is approved for clinical use—dexrazoxane (DEX, ICRF-187). Uniqueness of DEX corresponds to limited understanding of underlying mechanism(s) but at the same time it could serve as a key to uncover them. DEX is known to affect topoisomerase II (TOP2) enzyme and this enzyme is currently suspected to have an essential part in the development of ANT cardiotoxicity. To investigate this idea further, series of structurally different compounds described in literature as TOP2 inhibitors was selected and their effects on both human TOP2 isoforms were examined. Also, their own toxicity together with ability to prevent daunorubicin toxicity on well-established model of neonatal rat cardiomyocytes with good predictive value for subsequent in vivo chronic experiments. The work on clarification of mechanisms involved in development of ANT cardiotoxicity is still in progress, nevertheless present results of TOP2 inhibitors support the notion of TOP2 involvement but also indicate marked differences between these inhibitors and the mode in which they might affect function of the enzyme (apart from possible involvement of other potential targets of these compounds). Studies narrowing the broad range of structures from this screening into more delicate structure-activity investigation of promising lead compounds and their analogues are underway.

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INTERACTION OF CATECHOL DERIVATIVES WITH ESTROGEN RECEPTORS. CYTOTOXICITY AND GENE EXPRESSION

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Catechols and their derivates are widely present in diet (e.g., wine, tea, chocolate, fruit, and vegetables) but also as pollutants, for instance as components of cigarette smoke[1]. These compounds can be also found in humans as products of the metabolism of exogenous aromatic compounds including flavonoids or the degradation of endogenous neurotransmitters (noradrenaline, dopamine etc.)[2,3]. Estrogen receptor (ER, NR3A) is a nuclear receptor widely expressed in the human body able to interact not only with estrogens but also with other endogenous compounds and xenobiotics with different chemical structures. In this project, we evaluated the interaction of a total of 22 catechol derivatives with ER using the MCF-7 breast cancer cell line and its fulvestrant resistant derivate (MCF-7/182R-6). In addition, the cytotoxicity of the compounds was tested. Our results show, that 18 from the 22 tested compounds were not cytotoxic on the even at a concentration of 100 μM. ER interaction with catechol derivates was studied through the expression of its target TFF1 and ESR1 mRNAs after a 48-h exposure. Our results show that most of the studied derivates were able to enhance ER target genes transcription at a concentration of 10 μM.

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BPT 8

NOVEL ANTI-AML DRUGS AS INHIBITORS OF ABC TRANSPORTERS

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Despite the advances in understanding the biology of acute myeloid leukemia (AML), poor treatment outcome and low survival rate of patients remains an issue. However, drug resistance is a significant problem in the long-term treatment of AML. One of the primary mechanisms of resistance in anthracycline-treated AML is the drug efflux through ATP-binding cassette (ABC) transporters. Recently, new anti-AML drugs were developed and introduced to potentially participate in pharmacokinetic drug interactions and modulate multidrug resistance (MDR). Our studies started off with several of these novel and investigational drugs comprising glasdegib, edicotinib, bemcentinib evaluating their inhibitory effect on ABC transporters and evaluate the role in MDR. First, in accumulation assays in MDCKII cell lines with hoechst as a fluorescence substrate our results showed that glasdegib and edicotinib inhibits ABCG2 transporter, this was further confirmed by doing accumulations in HL60 cell lines with mitoxantrone as a substrate. XTT proliferation assay, performed on A431 cell lines subsequently demonstrated glasdegib that can reverse mitoxantrone resistance mediated by ABCG2. Lastly, the mRNA level of ABCB1, ABCG2 and ABCC1 were not influenced after 24 h exposure to glasdegib in LS174T cells, whereas bemcentinib was revealed as ABCG2 inducer. To conclude, glasdegib seems to be to be promising drugs in terms of transporter mediated MDR worthy further evaluations in AML cell lines and peripheral blood mononuclear cells isolated from *de novo* diagnosed AML patients.

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BPT 9

ON THE ROLES OF UGT1A1 AND UGT2B7 DRUG METABOLIZING ENZYMES IN CELLULAR CYTOSTATIC RESISTANCE

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Metabolic deactivation by UDP-glucuronosyltransferases (UGTs) is considered a potential mechanism of anticancer drug resistance. However, this hypothesis is predominantly based on indirect evidence and/or is influenced by interfering factors such as the use of multienzymatic models. Thus, an experimental approach for its verification is needed. In the present work, we employed HepG2 cells transduced with UGT1A1 or UGT2B7 enzymes, which are involved in epirubicin, etoposide and SN-38 metabolism, to provide mechanistic evidence on their possible roles in resistance to these chemotherapeutic drugs. Using CellTiter-Glo® viability assay, we showed that overexpression of UGT1A1 and UGT2B7 resulted in the decreased antiproliferative activities of etoposide/SN-38 and etoposide/epirubicin, respectively. In addition, the sensitivities of UGT1A1- and UGT2B7-transduced cells toward tested cytostatics were restored by the co-administration of model inhibitors, atazanavir and diclofenac, respectively. Our data demonstrate that metabolic deactivation mediated by UGT1A1 and UGT2B7 might be a crucial pharmacokinetic resistance mechanism for examined anticancer agents. Apoptosis studies and assays with proliferation-competent hepatocytes will be next employed to investigate this issue in detail.

The study was supported by the Czech Science Foundation (grant No. 20-20414Y) and by Charles University (SVV/2021/260-549)

BPT 10

ROLES OF CANCER STEM CELL – AND APOPTOSIS-RELATED PATHWAYS IN THE DEVELOPMENT OF ACQUIRED RESISTANCE TO EGFR INHIBITORS

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Non-small cell lung cancer (NSCLC) is the deadliest cancer type in both sexes. Epidermal growth factor receptor–tyrosine kinase inhibitors (EGFR-TKis) have been widely applied in the clinical treatment of NSCLC in the last two decades. Nevertheless, drug resistance emerging due to the long-term administration greatly limits the effectiveness of these drugs. Apart from mutations of the target, deregulation of oncogenic/tumor suppressor pathway represents a main factor contributing to this phenomenon. In this study, we aimed to elucidate the role of cancer stem cell- and apoptosis-related pathways in the development of EGFR-TKis resistance in NSCLC. First, we established HCC827 and NCI-H1975 cell lines resistant to gefitinib and dacomitinib/osimertinib, respectively. Using qRT-PCR and/or western blotting, we found that Bcl-2 level was abnormally increased in all three different drug resistant sublines, compared with their parental cells. The upregulation of Hedgehog and Notch pathways were found both in dacomitinib and osimertinib resistant cells. In addition, the results from MTT assay showed that gefitinib/dacomitinib/osimertinib resistant cell line has cross-resistant reactivity to erlotinib/afatinib/olmutinib, respectively. To sum up, our results suggested that cancer stem cells-related (Hedgehog and Notch) pathways as well as anti-apoptotic protein Bcl-2 can represent universal factors participating in the development of EGFR-TKis resistance. In the follow-up studies, we will evaluate whether these factors might be targeted by siRNA or pharmacological modulators. In addition, global proteomic analysis will be employed to reveal other proteins/pathways, which might play significant role in EGFR-TKIs resistance. Following *in vivo* confirmation, our results might provide an important information for clinicians to optimize the NSCLC therapy with EGFR-TKIs.

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BPT 11

ALISERTIB ACTS AS A DUAL ACTIVITY RESISTANCE MODULATOR THROUGH THE INHIBITION OF ABCC1 TRANSPORTER

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Alisertib (MLN8237) is a novel Aurora A kinase inhibitor, which has shown potent antitumor activity in clinical trials. In this work, we investigated possible interactions of alisertib with ABC drug efflux transporters and described its role in multidrug resistance (MDR). In accumulation studies, alisertib effectively inhibited the ABCC1 transporter, but there were no significant effects on ABCB1 or ABCG2. Drug combination studies confirmed the alisertib's role as a dual activity modulator; the drug synergistically enhanced antiproliferative effect of daunorubicin in ABCC1-overexpressing cells. The presence of MDR-associated ABC transporters did not impair the anticancer efficacy of alisertib, even though it acted as ABCB1 substrate in cell monolayer transport experiments. Thus, the possible victim role of alisertib in MDR was refuted. In addition, alisertib had no significant effects on the mRNA-level expression of tested ABC transporters in various cellular models. In conclusion, our data introduce alisertib as an effective dual activity modulator whose MDR-combating properties are not compromised by efflux or effect on MDR phenotype. This information might be beneficial for oncologists when introducing alisertib into the clinical area.

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BPT 12

PHOTOBIOLOGICAL PROPERTIES OF THE MOST ACTIVE PHTHALOCYANINES FROM INTERNATIONAL COOPERATION AS PHOTOSENSITIZERS FOR PHOTODYNAMIC THERAPY

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Phthalocyanines (Pc) are interesting compounds with exceptional photochemical and photophysical properties and has proven themselves as very promising second-generation photosensitizers for photodynamic therapy. This work is a part of the extensive international project in which we study the most active compounds from renowned foreign scientific groups under identical experimental conditions. We have focused on the series of Pcs derivatives and investigated their photophysical, physicochemical and biological properties with the aim of finding the factors that may contribute to the significant differences in the activity between Pcs bearing various peripheral substituents (cationic × anionic × non-charged, hydrophilic × amphiphilic).[1] Photodynamic activity was demonstrated *in vitro* on several cell lines (HeLa, MCF-7, SK-MEL-28) and were performed the comparative study in terms of cellular uptake and intracellular distribution, as well as their dark and photoinduced toxicity.

The study was supported by the Czech Science Foundation (19-14758Y) and Charles University (PRIMUS/20/SCI/013, GAUK 1620219).

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CRISPR/CAS9-MEDIATED KNOCKOUT OF *LRP2* GENE IN CELL LINES

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Low-Density Lipoprotein Receptor-Related Protein 2 (LRP2, megalin) is localized in many different tissues including kidney, where it is responsible for internalization of filtered molecules into the proximal tubular cells. Proteins (e.g. albumin), apolipoproteins or some antibiotics (e.g. aminoglycosides) are known to be recaptured by LRP2.[1] Ligands are transported by endocytosis, which can lead to lysosomal degradation of transferred substances and cause nephrotoxicity.[2]

Human cell lines HK-2 and JEG-3 expressing high level of megalin were used for editing of LRP2 knockout cell lines using CRISPR/Cas9 technique. sgRNA sequences were specifically designed to harm the important site for regulation of function and trafficking ligands (NPMY motif), or site which plays the central role in megalin phosphorylation (PPPSP motif) or transmembrane domain of LRP2.[1] Fluorescence-Activated Cell Sorting was involved to sort cells exerting low activity of megalin. The function of LRP2 in sorted cells was further verified experimentally using two methods.

Within the first method lower accumulation of LRP2 substrate fluorescent labeled FITC-albumin was determined. The second selected approach was to validate modified cells by change in viability after treatment with aminoglycoside antibiotic gentamicin, another LRP2 ligand, used at cytotoxic concentration.

Knockout of *LRP2* in transfected cell lines was confirmed by qRT-PCR.

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BPT 15

EFFECT OF PRO-INFLAMMATORY CYTOKINES ON ABC AND SLC TRANSPORTERS` GENE EXPRESSION IN HUMAN PLACENTAL CELL LINES AND VILLOUS EXPLANTS

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Membrane transporters located in the placental tissue play an important role in the transport of endogenous or exogenous substrates and thereby in the nutrition as well as protection of developing fetus. Normal labor is controlled by inflammatory signaling, and this process is a triggering mechanism that can be modified by internal stimuli, including infection and inflammatory stressors. Untimely onset of this process can lead to pregnancy complications including preterm birth. The aim of our present work was to investigate the possible effect of inflammation on the expression of selected SLC transporters using human first trimester trophoblast cell line HTR-8/SVneo, choriocarcinoma cell line BeWo and human term placental villous explants. Using real-time qRT-PCR, different expression pattern was observed following proinflammatory stimulation in all placental models. Following exposure of the choriocarcinoma BeWo cells to INFγ we observed increased expression of OATP2A1 transporter, encoded by *SLCO2A1* gene. Interestingly, placental villous explants demonstrated increase in the expression of *SLC22A4* and *SLCO3A1* encoding OCTN1 and OATP3A1 transporters, respectively, after being exposed to TNFα and LPS as pro-inflammatory stimulants. Changes in the expression of genes encoding placental SLC transporters was observed also in the first trimester HTR-8/SVneo cells. These results indicate that proinflammatory environment affects expression of membrane transporters and can thereby affect placental transport function and possibly fetal development as well as labor onset.

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BPT 16

TISSUE AND SOLUBLE ENDOGLIN CHANGES DURING LIVER FIBROSIS IN MICE

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Liver fibrosis is the consequence of chronic liver injury. Endoglin (CD105) is a 180 kDa transmembrane glycoprotein and a coreceptor for binding to TGFβ1 superfamily existing in two forms, namely membrane endoglin (Eng) and soluble endoglin (sEng) circulating in the blood. It has been demonstrated that Eng might play an important role in the process of liver fibrosis, inflammation, and endothelial dysfunction. However, the precise impact of changing in Eng expression, signaling, and sEng levels during these pathological changes in the liver are still unknown. The goal of this study was to analyze the expression of Eng and sEng levels with respect to biomarkers of inflammation, fibrosis and endothelial dysfunction in mouse model of liver injury. The liver damage was induced in three-month-old C57BL/6 female mice by DDC diet (3,5-diethoxycarbonyl-1,4-dihydrocollidine diet) while control animals were received standard chow diet, following by sacrificing the mice after four weeks with subsequent blood collection and molecular analysis of liver samples. Significant increase in the level of ALT, ALP, AST and total bilirubin ($P<0.01$) confirmed the liver impairment in the DDC mice. DDC diet significantly increased sEng levels and MMP-14 expression, however there was a significant reduction in the expression of Eng in liver ($P<0.01$). In contrast, significant increases in the expression of α-SMA and CRIP2 were observed in DDC treated mice ($P<0.01$). We suggest that DDC treatment results in cleavage of Eng by MMP-14 leading to high levels of sEng. Thus, sEng might be considered a circulating biomarker of liver damage after DDC treatment. However, the precise role of Eng expression changes with respect to the inflammation, fibrosis and endothelial dysfunction is currently under investigation in our lab.

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BPT 17

EFFECTS OF ANTI-ENDOGLIN ANTIBODY (TRC105) ON ENDOGLIN EXPRESSION, SIGNALING AND FUNCTION IN 7-KETOCHOLESTEROL AND HIGH GLUCOSE INDUCED ENDOTHELIAL DYSFUNCTION

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Endoglin (Eng) is the transforming growth factor β co-receptor that plays an important role in endothelial dysfunction and TRC105 is an antibody that blocks Eng and its downstream signaling. Here we investigated for the first time, the effects of TRC105 on the Eng expression, signaling, and function in 7-ketocholesterol (7K) and high glucose (HG) induced endothelial dysfunction. In the 7K study, human aortic endothelial cells (HAECs) were treated with TRC105 (300 μg/mL) for 1 hour, followed by the addition of 7K (10 μg/mL) for another 12 hours. In the HG study, HAECs were exposed to HG (45 mM) for 60 hours, followed by the addition of TRC105 (300 μg/mL) for another 12 hours, and cells treated with 5mM glucose and 40 mM mannitol served as osmotic control. Protein levels, adhesion, and transmigration of monocytes were assessed by flow cytometry, mRNA expression was measured by qRT-PCR. 7K and HG treatment increased protein levels of Eng and NF-κB, as well as adhesion and transmigration of monocytes through HAECs monolayer. TRC105 treatment reduced the 7K or HG induced Eng protein levels and Smad signaling. Despite increased protein levels of cell adhesion molecules (P-selectin and VCAM-1), TRC105-mediated blockage of Eng, prevented 7K and HG induced adhesion and transmigration of monocytes through endothelial monolayers. These results suggest that TRC105-mediated Eng blockage can counteract the 7K and HG induced endothelial dysfunction in HAECs, suggesting that Eng might be a potential therapeutic target in disorders associated with elevated cholesterol and glucose levels.

BPT 18

SOLUBLE ENDOGLIN AND LABETALOL AGGRAVATES ESTROGEN-INDUCED INTRAHEPATIC CHOLESTASIS

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Bile acids (BA) accumulated in plasma in pathological conditions leads to systemic toxicity. Intrahepatic cholestasis of pregnancy (ICP) and preeclampsia are serious comorbidities that threaten mothers and fetuses with adverse outcomes such as stillbirth or preterm delivery. Soluble endoglin (sEng) is a recognized biomarker for preeclampsia progression. Labetalol, an alpha-/beta-adrenergic receptor antagonist is the recommended antihypertensive therapy in preeclampsia, however this treatment was linked to hepatoxicity and ICP. Therefore, we aimed to elucidate the effect of labetalol therapy and increased sEng levels on BA metabolism in ICP animal model. Four-month-old wild-type female mice and transgenic mice overexpressing human sEng were used. Mice were administrated with ethinylestradiol (EE) (10 mg/kg s.c.) for 5 days (estrogen-induced intrahepatic cholestasis model), and/or labetalol (10 mg/kg s.c.) to simulate ICP and antihypertensive treatment in pregnancy. Expression of hepatic transporters was assessed by qRT-PCR and Western blot. Concentrations of BA were measured by LC-MS. We showed that labetalol treatment aggravated ICP systemic toxicity by increasing BA levels in plasma via Mrp4 upregulation, as well as increased circulating levels of sEng. In addition, high levels of sEng aggravated cholestasis in labetalol-treated mice with ICP. These data demonstrate that both labetalol and sEng aggravates ICP, and results in increased systemic exposure to BA predisposing mother and fetus for adverse pregnancy outcome. Thus, these data suggest the importance of monitoring sEng and BA concentrations in plasma of pregnant woman prone to preeclampsia and/or cholestasis.

The study was supported by GAUK No.1166119 and SVV 260 414

BPT 19

CONSTITUTIVE EXPRESSION OF SDR GENES IN DRUG-SUSCEPTIBLE AND DRUG-RESISTANT STRAINS OF *HAEMONCHUS CONTORTUS*

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Anthelmintic therapy in the agricultural industry is complicated by decreased drug efficacy. The effect of drugs can be reduced by the activity of parasites drug-metabolizing enzymes (DMEs). Previous studies have shown that increased anthelmintics inactivation via biotransformation belongs to a significant mechanism of drug-resistance in *Haemonchus contortus*, a gastrointestinal nematode of small ruminants. In resistant isolates, the increased expression of DMEs, e.g. cytochromes P450, UDP-glycosyltransferases and P-glycoprotein transporters, have been described. However, no information is available about other DMEs such as short-chain dehydrogenases/reductases (SDRs).

The relatively high number of genes from the SDRs class in *Haemonchus contortus* indicates the importance of these enzymes which could participate in biotransformation of carbonyl-containing anthelmintics (e.g. flubendazole and mebendazole) in *Haemonchus contortus*.

In present study, the constitutive expression of SDRs was analyzed and compared between drug-susceptible and drug-resistant strain of *Haemonchus contortus* and among various developmental stages (eggs, larvae, adults). The results showed the most expressed genes: SDR1 SDR3, SDR5 and SDR18. Expression of SDR12 was significantly different between both strains almost in all life stages. These SDR members will be studied further in view of their potential participation in flubendazole and mebendazole resistance in *Haemonchus contortus*.

The study was supported by the Charles University, project GA UK No. 194421

BPT 20

AUTOMATED MOTILITY ASSAY OF HAEMONCHUS CONTORTUS USING IMAGE RECOGNITION BASED ON DEEP LEARNING

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The parasitic nematode *Haemonchus contortus*, often known as barber's pole worm, is a useful model organism for drug screening of potential novel anthelmintics and it is also used in drug resistance studies. The current gold standard for measuring drug effectiveness is the worm motility assay for some larval stages. At present, the evaluation of motility assays is mainly manual, therefore time-intensive and difficult to scale. However, recent advancements in computer vision, specifically Convolutional Neural Networks (CNN), have reached a level where individual instances in an image can be identified with a very high degree of precision, including complex heterogeneous shapes. These algorithms allow us to potentially automate the process and create high-throughput systems. Therefore, we applied a state-of-the-art method Mask R-CNN[1] to analyze the motility of larvae within videos and compared the performance to other automated approaches and to manual processing in order to evaluate the precision and potential of new CNN algorithms.

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BPT 21

NEW POTENTIAL ANTHELMINTICS AGAINST *HAEMONCHUS CONTORTUS* - BLK127 AND HBK4: TOXICITY, EFFICACY, AND BIOTRANSFORMATION

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Haemonchus contortus is one of the most dangerous trichostrongiloid nematode of small ruminants with world-wide distribution. Due to its genetic diversity was able to develop resistance to all available anthelmintics. For this reason, new active compounds are highly needed. The phenotypic screening of compound library called 'Kurz Box' revealed two compounds designated as **BLK127** and **HBK4** which were active against larval stages of *H. contortus*. In the present study we asses activity of those compounds on adults and eggs of *H. contortus*, their hepatotoxicity and biotransformation. While none of the tested compounds had any effect on eggs hatching, BLK127 significantly decreased viability of *H. contortus* adults and was nontoxic in ovine and rat liver. Contrary, HBK4 didn't showed significant effect on viability of *H. contortus* adults and exhibited significant hepatotoxicity. These findings suggest BLK127 for further testing as new potential anthelmintic. Consequently performed biotransformation study led to identificaion of three main metabolic pathways of BLK127 in *H. contortus* adults: hydroxylation, hydrolysis and glucosidation.

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

BPT 22

EFFLUX TRANSPORTERS OF HAEMONCHUS CONTORTUS AND THEIR ROLE IN RESISTANCE DEVELOPMENT

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Efflux transporters like P-glycoprotein (P-gp) are involved in the transport of structurally unrelated xenotoxins, and have been recognized as major players in resistance to drugs in mammals, bacteria and parasites. Despite, a little is known about efflux transporters in helminths, P-gps were linked with anthelmintic resistance several times. *Haemonchus contortus*, a sheep parasite, pose a threat mainly due to its remarkable ability to become resistant. In this project by exposing *H. contortus* to various anthelmintic we investigate the role of P-gps in resistance. Looking into the expression of P-gp genes in all *Haemonchus* life stages and drug resistant and sensitive strains, we discovered significantly increased expression of Hco-pgp-9.2 in most cases. Therefore, we aim to characterize this transporter, to define the transport activity of Hco-pgp-9.2 and its interaction with substrates and inhibitors, Rhodamine efflux assay and ATPase activity measurement will be used. Currently, we focus on the localization of the Hco-pgp-9.2 gene by RNA in situ hybridization. The findings could bring more clarity to the mechanisms of resistance in *H. contortus* and nematodes in general and be useful in development of more efficient treatment in the future.

The study was supported by the Charles University foundation, grant No. 1171620.

BPT 23

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

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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 and protection from the toxic action. Our model organism is *Haemonchus contortus*, a gastrointestinal parasite of small ruminants that have a remarkable ability to develop resistance to anthelmintic drugs. The involvement of UGTs in resistance caused by biotransformation of anthelmintics was confirmed by observed differences in glycosides quantities between resistant and sensitive strains.

The activity of UGT enzymes was measured in subcellular fractions of *H. contortus* by the LC-MS. Anthelmintic – albendazole was used as substrate for UGTs and we have detected several glycosides. Furthermore, we have investigated the involvement of UGTs by using several known UGT inhibitors (sulfinpyrazone, 4,6-dihydroxy-5-nitropyrimidine, 5´nitrouracil). The sulfinpyrazone in different concentrations reduced the enzyme activity and formation of glycosides. We believe UGTs can be further exploited as molecular targets for combination therapy.

BPT 24

BIOCHEMICAL ASSAY FOR VIABILITY TESTING IN SUSCEPTIBLE AND RESISTANT STAINS OF *HAEMONCHUS CONTORTUS*

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The parasitic nematode *Haemonchus contortus* is a highly prevalent gastrointestinal parasite of small ruminants. Treatment with chemical drugs is the weapon of choice, however, the efficacy of all anthelmintics is hampered due to widespread drug resistance. Therefore, there is a necessity for viability assays in *H. contortus* not only for detection of drug resistance but also for testing of novel drugs for their potential anthelmintic effect. The current approaches comprise of using microscopic techniques to observe viability in predominantly free living stages, however, they are very time-consuming. To address the missing biochemical assays of viability testing in adults, the target stage of the anthelmintic treatment, we adopted the bioluminescence assay of adenosine triphosphate. The optimized protocol was used to test the effect of levamisole on exsheathed larvae from drug-susceptible and the drug-resistant strains. We also tested the effect of levamisole and monepantel on females and males. According to our results, the ATP assay has expanded the possible assays not only for viability testing, but also for the detection of drug-resistant isolates, and it is the first biochemical assay in the adult stage.

The study was supported by UNCE 18/SCI/012, GAUK 1568519, and SVV 260 550 as well as by the project EFSA-CDN (CZ.02.1.01/0.0/0.0/16_019/0000841), co-funded by ERDF.

BPT 25

IN VITRO FORMATION OF DUAL-SPECIES BIOFILMS – ESTABLISHMENT OF EXPERIMENTAL SETTINGS FOR METHOD LEADING TO RECOGNITION OF ANTI-BIOFILM ACTING COMPOUNDS

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Currently, it is known that microorganisms, including multidrug-resistant ones, prefer a lifestyle in complex communities called biofilms. These complex consortia provide their participants more protection against hostile environmental factors, such as antimicrobials or host defense mechanisms. This is reflected in the fact that biofilm-associated infections are mainly linked with a chronic course of diseases, and the treatment of these infections is challenging. It has been demonstrated that mutual interactions among biofilm-forming microbes can be reflected in the enhancement of antimicrobial resistance.[1] One of the pitfalls in anti-infective drug discovery research also lies in the low correlation between results of the candidate compounds activity *in vitro* compared to results obtained from the clinical trials.[2] To reflect this potential gap, we are focused on the implementation of methodological approaches leading to robust dual-species biofilm biomass formation *in vitro*. Microorganisms colonizing wounds or venous catheters, namely *Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa,* and *Candida albicans* were chosen for the study. For the establishment of the optimal conditions for biofilm formation *in vitro,* parameters such as the viability, quantitative representation of individual species, a quantity of the biofilm matrix, *etc*., are evaluated.

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BPT 26

ALDO-KETO REDUCTASE 1C1 (AKR1C1) MODULATES GLUCOCORTICOID RECEPTOR ACTIVITY BY A PROTEIN-PROTEIN INTERACTION

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Corticosteroids (CS) are steroid hormones contributing to homeostasis in almost all tissues, and their use remains among the best cost-effective anti-inflammatory therapies. CS mainly exert their functions by binding to the glucocorticoid receptor (GR), which translocates to the nucleus to promote the assembly of multiprotein regulatory complexes exerting transcriptional activation or repression of glucocorticoid responsive elements (GREs). In this study, we evaluated the influence of the Aldo-Keto Reductase Family 1-member C1, C2, and C3 on the anti-inflammatory activity of dexamethasone *in vitro*. Using respiratory epithelial cell lines with silencing or overexpression of AKR1C1-3, we deciphered AKR1C1 and AKR1C2 as negative regulators of the GR-dexamethasone transcriptional activity reported by a GRE-luciferase screening system. This also correlated with alterations in the phosphorylation status of ERK1/2 and the gene expression under LPS induced-inflammatory response. Furthermore, confocal immunofluorescence and co-immunoprecipitation studies showed how AKR1C1 and GR co-localize in the nucleus in a dose-dependent manner. In conclusion, we propose AKR1C1 as a potential coregulator protein binding to the GR and modulating its transcriptional activity.

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BPT 27

ZANUBRUTINIB AS A NOVEL INHIBITOR OF ALDO-KETOREDUCTASE 1C3 FIGHTS AGAINST RESISTANCE IN DAUNORUBICIN CANCER THERAPY

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Anthracyclines are widely used in oncological practice due to their high efficacy. However, their usage is accompanied by the emergence of resistance and is limited by severe adverse effects, especially cardiotoxicity. Besides other mechanisms, resistance is mediated through the activity of carbonyl-reducing enzymes (CREs) from the superfamily of aldo-ketoreductases (AKR) and short-chain dehydrogenases/reductases (SDR). AKR1C3, along with some other CREs, reduce the hydroxyl groups of anthracyclines to secondary alcohols generating metabolites with impaired pharmacological effects. The inhibition of CREs is considered a promising approach to fight against resistance and cardiotoxicity. Tyrosine kinase inhibitors are modern anticancer agents that have shown additional targeting of proteins involved in cancer multidrug resistance, such as membrane efflux transporters and detoxifying enzymes. Zanubrutinib is a next-generation Bruton tyrosine kinase inhibitor approved to treat mantle cell lymphoma. Our project aims to investigate the ability of zanubrutinib to inhibit selected human CREs and potentiate the effect of daunorubicin. To accomplish our goals, we used incubation methods with recombinant enzymes, measuring inhibition in intact cells, and drug combination assays. The achieved results confirmed the ability of zanubrutinib to inhibit the AKR1C3 enzyme at cellular level and synergistically potentiate the antiproliferative effect of daunorubicin. In conclusion, the combination of daunorubicin together with zanubrutinib might become an effective approach for the prevention of anthracycline resistance in clinical practice.

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BPT 28

OFF-TARGET LIPID METABOLISM DISRUPTION BY THE MOUSE CONSTITUTIVE ANDROSTANE RECEPTOR LIGAND TCPOBOP IN HUMANIZED MICE

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The constitutive androstane receptor (CAR) controls xenobiotic clearance and regulates liver energy homeostasis, but these functions have been discovered using mouse-specific CAR ligand TCPOBOP in wild-type or CAR null mice. However, TCPOBOP treatment results in off-target metabolic effects in CAR null mice. In this study, we compared wild-type and humanized CAR-PXR-CYP3A4/3A7 mice treated with TCPOBOP. In this model, human CAR retains its constitutive activity in metabolism regulation; however, it is not activated by TCPOBOB. Notably, we observed that TCPOBOP affected lipid homeostasis and promoted hepatocyte hypertrophy in humanized mice. Hepatic lipidomic analysis revealed a significant accumulation of triglycerides in humanized CAR mice. Gene expression regulation in humanized mice is mainly involved in lipid metabolic processes and in the PPAR, leptin, thyroid, and circadian clock pathways. In contrast, CAR activation by TCPOBOP in wild-type mice reduced liver and plasma triglyceride levels and induced a typical transcriptomic proliferative response in the liver. In summary, we identified TCPOBOP as a disruptor of lipid metabolism in humanized CAR mice.[1]

The study was supported by GACR (No. 19-14497S, Czech Republic) to P.P.

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BPT 29

EXPRESSION DYNAMICS OF PREGNANE X RECEPTOR-CONTROLLED GENES IN 3D PRIMARY HUMAN HEPATOCYTE SPHEROIDS

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The pregnane X receptor (PXR, *NR1I2*) is a liver-enriched transcription factor, which is activated by a variety of ligands including rifampicin, one of the most clear-cut ligands of human PXR.

3D spheroids of primary human hepatocytes (PHHs) represent *in vivo* relevant hepatocyte model, which supports long-term experiments due to its stable phenotype.

Of note, PXR controls hepatocyte expression of numerous genes, however, the temporal dynamics of PXR target gene expression is largely unknown.

To this end, we employed 3D PHHs to provide time-dependent expression profiles of 12 prototypic PXR-controlled genes (CYP3A4, CYP2C9, CYP2B6, MDR1, FASN, GLUT2, G6PC, PCK1, CYP7A1, SHP, PDK4, and PXR) in the time course of 168 h of rifampicin treatment (1 or 10 μM). To our knowledge, this is the first attempt to cover time profiles of PXR action with high temporal resolution in PHH spheroids.

In my talk, I will show data confirming basal expression stability of PXR-controlled genes in 3D PHHs. Further, I will discuss rifampicin-induced gene expression patterns to emphasize that one point or short-term observations may lead to misleading conclusions regarding the biological impact of PXR activation.

Overall, our work demonstrates the importance of long-term time-expression profiling of PXR target genes and provides insight into PXR function in liver beyond our knowledge from conventional 2D *in vitro* models.

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BPT 30

CHANGES IN THE EXPRESSION OF PHASE I DRUG-METABOLIZING ENZYMES IN TWO MOUSE MODELS OF NAFLD

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Non-alcoholic fatty liver disease (NAFLD) is a chronic liver disease with increasing worldwide prevalence. NAFLD is an umbrella term that covers a spectrum of hepatic conditions varying in severity of injury and resulting fibrosis. Non-alcoholic steatohepatitis (NASH), a more serious form of NAFLD, is characterized by the presence of hepatic steatosis, inflammation, and hepatocyte damage. In addition, NASH may further progress into advanced fibrosis, hepatic cirrhosis, hepatocellular carcinoma, and eventually liver failure. As liver plays a central role in the metabolism of xenobiotics, the aim of the present study was to characterize the effect of NAFLD on the expression of phase I drug-metabolizing enzymes (DME). For this purpose, two mouse models of NAFLD – a dietary model and a chemically induced model, were used. Real-time quantitative polymerase chain reaction (RT-qPCR) and western blotting were employed to study the changes in the expression of DMEs. NAFLD significantly altered the expression of several cytochrome P450 (CYP) and aldo-keto reductase (AKR) enzymes. Moreover, the changes differed between the two mouse models of NAFLD. In the dietary model, the mRNA expression of CYP1A1/2, CYP2B, AKR1A, AKR1C6, and AKR1C20 was increased, while only the expression of CYP3A11 was decreased. In the chemically induced model, which typically represents a more severe form of NASH, the mRNA expression of CYP2B, AKR1C6, and AKR1C20 was increased, but the mRNA expression of other DMEs, namely CYP1A1/2, CYP3A13, CYP3A11, and AKR1A was found to be decreased.

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BPT 31

EFFECT OF NAFLD ON THE EXPRESSION OF ANTIOXIDANT ENZYMES

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Non-alcoholic fatty liver disease (NAFLD) is a multifactorial and complex disease associated with obesity, insulin resistance, metabolic syndrome, and type II diabetes mellitus. NAFLD, which is characterized by the excessive fat accumulation in the liver, ranges from simple steatosis to steatohepatitis, advanced fibrosis, and cirrhosis with end-stage liver disease. Mitochondrial β-oxidation of free fatty acids and consequent formation of reactive oxygen species are enhanced in fat-overloaded hepatocytes. Progression of simple steatosis to more severe NAFLD stages is promoted by oxidative stress. Changes in the expression and/or activity of antioxidant enzymes protecting cells against oxidative stress during NAFLD has been reported. As NAFLD is becoming a major public health concern, objective of this study was to investigate NAFLD-induced changes in the expression of antioxidant enzymes at mRNA and protein level. Liver samples from control mice and monosodium glutamate-induced obese (MSG) mice both fed with standard or high-fat diet were obtained and the induction of steatosis was confirmed histologically. The mRNA and protein expression of glutathione S-transferase, glutathione peroxidase, glutathione reductase, thioredoxin reductase, superoxide dismutase, catalase, and NAD(P)H:quinone oxidoreductase were determined using real-time quantitative PCR and western blotting, respectively. NAFLD caused changes in the expression of some antioxidant enzymes on both levels in control as well as in MSG mice. The influence of NAFLD on a specific activity of these enzymes will be further studied.

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BPT 32

THE EFFECT OF NUCLEOSIDES ON TROPHOBLAST CELLS PROLIFERATION; PRELIMINARY DATA

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Placental trophoblast cells are equipped with a wide array of transporters and receptors that are crucial for their proper growth/tuning up the development of the placental barrier and nourishment of the developing fetus. Micromolar concentrations of nucleosides were detected in the placental blood supply and placental tissue. Keeping with that, trophoblasts express large amounts of nucleoside transporters (NTs) and adenosine receptors (ARs). However, the contribution of nucleosides, NTs, ARs, and the interplay between NTs and ARs to placental growth is not known. Therefore, we analyzed the effect of increasing adenosine (Ado), guanosine, and nucleoside cocktail containing uridine, thymidine, and cytosine on metabolic activity, DNA synthesis, and DNA content in trophoblast-derived BeWo and JEG-3 cell lines. Using qRT-PCR, we also tested whether Ado affects the gene expression of NTs and ARs. Ado and guanosine tested at physiological concentrations increased metabolic activity, amount, and synthesis of DNA, while the nucleoside cocktail did not exhibit any effect. The effect of Ado was further confirmed by increased levels of a proliferation marker MKI-67. Ado and guanosine induced a greater growth effect in BeWo cells compared to JEG-3 cells that exhibited expression of only AR_{A2A} and lower levels of the equilibrative nucleoside transporter 1. Supraphysiological Ado concentrations did not accelerate proliferation; however, surprisingly increased the expression of AR_{A2A} and NTs in BeWo cells. Furthermore, we observed that Ado does not increase syncytin levels, a marker of trophoblast differentiation, and the differentiation trigger, forskolin, does not influence AR mRNA levels in the BeWo cell line. We conclude that Ado and guanosine accelerate trophoblast proliferation by increased cellular uptake followed by facilitated DNA synthesis and/or activating AR_{A2A} and AR_{A2B}. We will continue the study by testing the effect of specific agonists/antagonists of ARs and inhibitors of NTs. We will also expand the study to explants, an ex vivo model of the placenta.

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BPT 33

ESTABLISHMENT OF AN *EX VIVO* PRECISION-CUT SLICE MODEL TO STUDY INFLAMMATORY RESPONSE IN RAT PLACENTA

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Inflammation exerts profound effects on pregnancy and causes several complications, including the increased risk of poor fetal brain development and programming. Current models used to study the inflammation are placental explants, primary trophoblast cells and immortalized cell lines. All these models have their own pros and cons; the common limitations of all these models are shorter life span and variation in the level of expression of genes and proteins. In order to design a new study model with a greater life span and recapitulate *in vivo* system, we have developed an *ex vivo* precision-cut slice model (with a life span of about five days) using rat term placenta. Inflammation was induced with LPS and Poly I:C at different concentrations for 4h and 18 h. We examined the expression of several pro-inflammatory genes and cytokine secretion by qPCR and ELISA, respectively. Our results showed considerable induction of IL-1β, TNF-α and IL-6 at both mRNA and protein levels. Interestingly, this effect was differential in male and female placentas. To establish translational relevance to our findings on the impact of LPS on the inflammatory response in rat slices, we tested several anti-inflammatory compounds and examined their abrogative effect of inflammatory response induced by LPS.

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BPT 34

A COMPREHENSIVE ASSESSMENT OF ASSOCIATIONS BETWEEN PRENATAL INFLAMMATION AND PLACENTAL EXPRESSION SIGNATURE OF TRYPTOPHAN METABOLISM IN PRETERM BIRTH

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Spontaneous preterm birth is a serious medical condition responsible for substantial perinatal morbidity and morbidity. Both preterm labor with intact membranes (PTL) and preterm premature rupture of the membranes (PPROM) are associated with significant neurological and behavioral changes in adulthood. In this study, we hypothesize that the hostile inflammatory environment associated with preterm delivery may lead to disturbed placental endocrine and transport functions. The expression signature of placental tryptophan metabolism, an important pathway in prenatal brain development and immunotolerance, was evaluated in a clinical cohort of healthy term (n = 39) and 167 preterm deliveries (PTL, n = 38 and PPROM, n = 129). Maternal serum C-reactive protein and white blood cell count, and amniotic fluid interleukin-6 levels, were investigated as inflammatory markers. Using robust statistical analysis, we reveal putative relationships between maternal inflammation, preterm delivery, the placental tryptophan pathway, and gestational age at delivery. Elucidating these associations will help understand the complex processes linking maternal/intrauterine inflammatory events and poor neurodevelopmental outcomes.

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BPT 35

TRYPTOPHAN METABOLISM IS MODULATED BY MATERNAL INFLAMMATION

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Maternal immune activation (MIA) during pregnancy constitutes a well-established risk factor for offspring neuropsychiatric disorders. While the intrauterine bacterial infection is a known risk factor for autism, the systemic maternal viral infection is a reported risk factor for autism and schizophrenia. 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. In this study, we evaluate the effect of bacterial (LPS) and viral (poly I:C) placental infection on TRP metabolism using an *ex vivo* model. Human placenta explants were exposed to LPS or Poly (I:C) for 4 or 18 hours; gene and protein expressions, as well as functional enzymatic activities of the main enzymes of TRP metabolism were evaluated. Our results confirm that several enzymes in TRP metabolism are differentially affected by inflammation. The first and rate-limiting enzyme for the serotonin pathway (TPH) decreased significantly (at protein and functional level) in explants exposed to bacterial infection (LPS). On the other hand, the rate-limiting enzyme for the kynurenine (KYN) pathway, IDO, increased significantly (gene expression and functional level), suggesting that inflammation promotes TRP metabolism mainly via the KYN pathway. Furthermore, within the KYN pathway, the KMO-related course was increased, suggesting that inflammatory mediated release of kynurenine metabolites from the placenta may expose the fetus to higher amounts of potentially neurotoxic molecules.

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BPT 36

DYNAMIC CHANGES IN MONOAMINE TRANSPORT UPON TROPHOBLAST DIFFERENTIATION; EVIDENCE FOR TRANSCRIPTIONAL AND FUNCTIONAL VARIABILITY BETWEEN PRIMARY HUMAN VILLOUS AND BEWO TROPHOBLASTS

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For proper fetal development and programming, tightly regulated levels of monoamines (serotonin, noradrenaline, and dopamine) must be maintained in the feto-placental unit during gestation. The placenta is equipped with a set of transporters and enzymes localized in the trophoblast cells; polarization of syncytiotrophoblasts (STBs) gives rise to the maternal-facing microvillous membrane (MVM) and fetal-facing basal membrane (BM). Functional expression of the serotonin transporter (SERT) and noradrenaline transporter (NET) has been observed in MVM, presence of organic cation transporter 3 (OCT3) is attributed to BM. Although alterations in transcriptomic profile have been observed in STBs and their precursors, cytotrophoblasts (CTBs), the effects of trophoblast differentiation on placental monoamine uptake is not known. Various experimental approaches have been employed to imitate the placental barrier, including in vitro cell cultures – primary human trophoblast (PHT) and BeWo. However, cancerous BeWo cells have often been questioned about their suitability for placental physiology research. By applying gene/protein expression and functional analyses we investigated the impact of trophoblast cell differentiation on monoamine transport in PHT and BeWo. Our study demonstrates the effect of trophoblast differentiation on monoamine uptake; in addition, we show that BeWo cells do not mimic the physiological condition of the placenta.

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BPT 37

EFFECT OF PRENATAL ANTIDEPRESSANT USE ON MONOAMINE HOMEOSTASIS IN THE FETO-PLACENTAL UNIT; TRANSCRIPTOMIC AND ULTRASOUND STUDIES

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Monoamines are crucial neuromodulators for proper placenta function and fetal development, including cell proliferation, differentiation and neuronal migration. Therefore, even a transient disruption of their balance may lead to lasting changes in the brain structure and function, projecting into adulthood. Placenta, despite being a non-neuronal organ, has a similar suite of transporters and enzymes to regulate monoamine levels in the fetoplacental unit. However, there is still a lack of information about placental handling of NA and DA. Therefore, in the present study, using the method of microvillous and basal membrane vesicles isolated from healthy human term placentas, we performed time-, concentration-, temperature- and inhibitor-dependent uptake of DA and NA. Our results indicate that the high-affinity and low-capacity transporters on MVM (SERT, NET) and low-affinity and high-capacity transporter on BM (OCT3) are responsible for placental monoamines uptake from maternal and fetal circulation. In addition, DA is partly transported also by passive diffusion on both MVM and BM.

Further, we studied acute effect of ADs on monoamine uptake using the method of microvillous and basal membrane vesicles isolated from human term placenta and *in situ* rat placenta perfusion. Results revealed paroxetine (PAR) being the most potent inhibitor of monoamine uptake. Therefore, in the follow up study, we investigated possible effects of chronical paroxetine (PAR) treatment on monoamine homeostasis in the placenta and fetal brain in Wistar rats. Moreover, using Doppler ultrasound, we examined the effect on fetoplacental perfusion. Doppler ultrasound results indicated increased uterine and umbilical pulsatility index in PAR-treated groups, indicating increased maternal and fetal circulation resistance. Moreover, treated groups had significantly smaller fetal and placental weights. Finally, we found significant alterations in gene expression of enzymes and transporters involved in monoamines homeostasis in the placentas and fetal brains. Our results demonstrate that chronic PAR use during pregnancy influences fetoplacental circulation and gene expression of monoamines homeostasis machinery, highlighting safety concerns about ADs treatment during pregnancy.

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