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Abstracts

Sections 1 and 4 - Biochemistry, Pharmacology and Toxicology

BPT1

IN VITRO TESTING OF SELECTED CATECHOLS AND PHENOLS AS POTENTIAL ENDOCRINE-DISRUPTING AGENTS

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The presence of molecules from the catechol and phenol class in the human body can be attributed to the metabolism of larger molecules found in food (e.g. berries, chocolate, beer, etc) (1). These compounds are considered of interest due to their potential pharmacological properties (antioxidant, anti-inflammatory, antiplatelet, etc.) (2, 3). In this project, we aimed to determine the *in vitro* estrogenicity of a total of 22 catechols and phenols, both of natural and synthetic origin. Experiments were performed using the estrogen receptor (ER)-positive MCF-7 cell line. Compounds showed good safety profiles since only 4 out of the 22 compounds (3-isopropylcatechol, 3,5-dichlorocatechol, 4,5-dichlorocatechol, and 4-nitrocatechol) showed minor toxicity, and only at the highest concentration tested (100 μM). Interaction of the compounds with ER was confirmed by studying gene expression of 2 ER-target genes (ESR1 and TFF1) by means of RT-PCR. Compounds were tested at concentrations 10 and 50 μM, and compared to estradiol, as positive control. A total of 7 compounds showed a similar genetic expression pattern as that of estradiol, indicating a significant interaction with the receptor. These results were confirmed by protein expression assays performed by Western-Blot, particularly for 3-methylcatechol (3-MC) and 3-nitrocatechol (3-NC). In summary, although most compounds were able to interact with ER, only 3-MC and 3-NC can interact with ER in a significant manner.

The study was supported by Charles University SVV 260 663

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BPT 2

ISORHAMNETIN AND TAMARIXETIN, COMPONENTS OF HAWTHORN, EXERT *EX VIVO* VASODILATORY EFFECTS IN PORCINE CORONARY ARTERY WHICH ARE MEDIATED BY L-TYPE CALCIUM CHANNELS

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Hawthorn produces clinically proven beneficial effects in cardiovascular diseases which may include vascular effects[1-3]. So far, the responsible compound and the mechanism of action is unknown. In this study, we aimed to investigate the possible vasoactivity of principal hawthorn constituents and its mechanism(s).

The *ex vivo* isolated tissue bath system (Krebs solution, 37°C, carbogen oxygenation, isometric measurement of tissue dilation/contraction) and porcine coronary artery precontracted by KCl (40 mM) were used for screening of principal constituents of the Hawthorn extract for vasoactive properties and these effects were compared to nitroprusside sodium (100 μM). In a separate set of experiments, the mechanisms of vasodilatory action of the two most active substances were determined using various activators/inhibitors and endothelium intact or denuded vessel rings.

Various constituents of the Hawthorn extract showed vasodilatory properties with isorhamnetin and tamarixetin being the most potent (EC_{50}= 47 and 48 μM, respectively). For both substances, the vasodilatory action is mediated by L-type of Ca^{2+} channels on the vascular smooth muscle and the effect is dose-dependent. In contrast, no significant effects on vascular guanylyl cyclase, PKG or PKA pathways, and various K^+ channels (BK_{Ca}, K_V, K_{IR}, K_{ATP}) were found.

The study was supported by Grant Agency of Charles University (GA UK), Project No. 236923

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BPT 3

EVALUATION OF THE NLRP3 INFLAMMASOME IN THE PLACENTA: MOLECULAR ACTIVATION BY LPS, GLUCOSE AND METFORMIN

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Inflammasomes are multiprotein complexes associated with pathogen clearance and sterile inflammation. Recent work has shown that the human placenta expresses high levels of NLRP3- inflammasome-associated molecules and secretes large amounts of proinflammatory cytokines IL-1β and IL-18. Interestingly, as a sterile inflammatory condition, gestational diabetes mellitus (GDM) is associated with elevated circulating levels of proinflammatory cytokines, including IL-1β. Moreover, metformin, a hypoglycemic agent used in pregnancy, has recently attracted interest in its potential to modulate inflammasome activation. Nevertheless, little is known about the placenta's NLRP3 characteristics or how it relates to metformin and GDM. Thus, we aimed to i) characterize the NLRP3 pathway in the human placenta (first trimester and term), ii) assess cellular expression in isolated primary trophoblasts and placental cell lines (BeWo, HTR-8/SVneo, and ACH-3P), and iii) evaluate the effects of LPS, high glucose and metformin on placental NLRP3 using villous placental explants isolated from human term placenta. qPCR and ELISA were used to evaluate the gene expression and cytokine release, respectively. We revealed differential expression of NLRP3 inflammasome-related components across gestation and in various placental cell lines. Moreover, we show a strong and modest effect of LPS and high glucose, respectively, on the placental NLRP3 pathway. Lastly, our ex vivo studies uncovered a potential proinflammatory effect of metformin in the placenta. Further research is necessary to identify mechanisms by which these agents exert their effects on the placental NLRP3 inflammasome.

The study was supported by the Grant Agency of Charles University (GAUK 170-050/235012) and the Czech Health Research Council (Grant number: NU22J-01-00066).

BPT 4

CHARACTERIZATION OF EX VIVO AND IN VITRO MODELS TO STUDY THE CROSSTALK BETWEEN THE ENDOCANNABINOID AND IMMUNE SYSTEMS IN THE PLACENTA

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The significance of the underappreciated endocannabinoid system (ECS) in reproductive biology—particularly in relation to placental health—is paramount. Maternal inflammation, linked to issues like preterm birth, emphasizes the significance of ECS. Despite known interactions with the immune system, the crosstalk between the ECS and placental inflammation remains poorly understood. Recognizing its contribution to the inflammatory imbalance is vital. Here, we hypothesized that the ECS, via its key endocannabinoids, 2-arachidonoylglycerol (2-AG) and anandamide (AEA), is crucial in modulating placental inflammatory responses. We initially employed qRT-PCR to analyze ECS gene expression in diverse placental models: explants from the human term placenta, homogenates from the first and third trimesters, isolated primary trophoblasts, and placental cell lines (JEG3, BeWo, HTR-8/SVneo, and ACH-3P). Additionally, placental explants were treated with various concentrations of AEA or 2-AG (0.1, 1, 10, and 20 μM) for 48 hours. After pre-treatment, we induced inflammation with 1 μg/ml lipopolysaccharide for 4 hours. Subsequently, we utilized qRT-PCR and ELISA techniques to assess the effects of 2-AG and AEA on gene/protein expression of the pro-inflammatory cytokines (TNF-α, IL-1β, IL-6, and IL-18). The study identified ECS gene expression in various placental models, revealing distinct differences. Endocannabinoids slightly reduced TNF-α and IL-1β levels in explants without affecting gene expression. Although more research is needed, the study advances the understanding of ECS in the human placenta and its interaction with the inflammatory milieu.

The study was supported by GAUK 170-050/235011, the Grant Agency of Charles University, GACR 23-07094S.

BPT 5

THE EFFECT OF DIFFERENT NUTRIENT CONDITIONS ON THE FORMATION OF DUAL-SPECIES METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* AND *CANDIDA ALBICANS* BIOFILM

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Biofilms are a significant clinical threat in rapidly increasing antimicrobial resistance and the underlying reason for most chronic, usually polymicrobial, wound infections. Especially thanks to the biofilm matrix, microorganisms are more resistant to hostile conditions and hitting them is much more complicated. Therefore, new effective anti-biofilm therapeutic strategies are critically needed.[1] To avoid falsely promising results in the basic research, this study aimed to

establish nutritional conditions leading to the formation of the community with the highest degree of typical biofilm attributes. Clinically relevant methicillin-resistant *Staphylococcus aureus* and *Candida albicans* dual-species biofilms were formed in four different cultivation media with various host effector molecules. To compare them, key biofilm attributes, such as total biofilm biomass, or the biofilm's ability to withstand exposure to selected antimicrobial drugs were evaluated. Next, the principal matrix biomolecules, or representation of individual microorganisms were quantified. The most appropriate nutrition conditions concerning the biofilm biomass structure, and the highest degree of tolerance to selected antimicrobial drugs with the evident contribution of the biofilm matrix, provides the Lubbock medium. Moreover, regardless of the nutritional conditions, carbohydrates were revealed as the prevailing matrix biomolecules, which could be targeted in the alternative antibiofilm strategy.

The study was supported by the Ministry of Health (CZE; NU21-05-00482) and SVV project No. 260 663.

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BPT 6

ANTHELMINTIC ACTIVITY OF ALBENDAZOLE ON *C. ELEGANS* WILD-TYPE AND IVERMECTIN-RESISTANT STRAINS

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Helminthiasis is a serious worldwide problem resulting in high human morbidity and economic losses in livestock and agriculture, especially in developing countries. For these reasons, control and reducing impacts remain a major priority in international public health and agriculture.

Anthelmintics are an effective defense against parasitic infections caused by helminths. Unfortunately, previous inaccurate and intensive use in livestock led to the evolution of resistance, which is now globally widespread for multiple parasitic nematode species of domestic animals. The similarity of the metabolic pathway through detoxification and transport enzymes of some anthelmintics can be a risk for the development of cross-resistance. Parasitic nematodes are very difficult to work with, requiring passage through their host for the maintenance of their parasitic life cycle. This complicates quantitative experiments in their natural habitat. Although, the lifestyle of the free-living worm *Caenorhabditis elegans* is very different from that of the parasites, it is probably safe to conclude, that *C. elegans* is no more dissimilar to parasitic nematodes than each individual species of parasite to another and has become a model organism for parasitic nematode research and great system for the screening of compounds with potential anthelmintic activity.

Previous studies suggest that BZMs affect many processes in *C. elegans*. In this study, we hypothesized from the idea that the ivermectin-resistant strain (IVR10) of *C. elegans* could be more tolerant to BZM compounds with a similar way of metabolization or transportation as macrocyclic lactones. The larval development assay and motility assay methods were used to verify the anthelmintic activity. The results of IVR10 were compared with the susceptible strain N2B to confirm or refute the presence of cross-resistance.

The study was supported by Erasmus+ (no. 3219340).

BPT 7

PURINE AND PYRIMIDINE METABOLIC PATHWAYS IN THE PLACENTAL BARRIER

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The placenta is essential for fetal development, facilitating substance exchange between maternal and fetal blood and maintaining a barrier to prevent direct blood mixing. Its growth resembles tumor characteristics but is a regulated process vital for fetal health. Nucleosides and nucleotides are crucial for cancer proliferation and immunotolerance. The placenta is equipped to take up, transfer, and metabolize nucleosides/nucleotides and to process adenosine signals. However, the dynamic changes during the placental growth and the role of nucleosides/nucleotides for the placenta's well-being have yet to be addressed. We demonstrated changes (predominantly upregulation) in expressions of the genes coding enzymes involved in de novo synthesis/salvage pathways of pyrimidine and purine nucleotides, adenosine metabolism, and signaling. It likely corresponds to the extensive growth of the placenta indicating that the increased capacity of the placenta at term for nucleoside maintenance and/or production. Amounts of purine metabolites measured were significantly different in preterm placentas. Interestingly adenosine correlated with the week of the birth, birth weight of preterm and term newborns fostering the influence of adenosine on pregnancy outcomes. Subsequently, we confirmed the metabolic data by gene expression analysis. Our data thus support the importance of adenosine and other nucleosides for the well-being of the placenta and likely proper fetal development. Our findings could be pivotal in elucidating potential placental causes of preterm births.

The study was supported by GACR 22- 17643S, GAUK 236523.

BPT 8

MULIPLE EFFECT OF ADENOSINE, OTHER NUCLEOSIDES, AND ANTIRETROVIRALS IN THE PLACENTAL TROPHOBLAST

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The placenta, an essential organ in fetal development, is known for its rapid, tumor-like growth. Nucleosides/nucleotides are key components involved in cancer cell proliferation and induction of immunotolerance, but their functions in the placenta have not been addressed so far. Antiviral therapies (ART) containing nucleoside analogs are linked to poorer pregnancy outcomes such as fetal growth restriction or preterm birth. Their possible interference with nucleoside/nucleotide homeostasis, however, has yet to be investigated . In the parallel study, we found that placental capacity to maintain levels of nucleotides increases towards term, and placentas collected from preterm births reveal altered levels of purine metabolites and gene expressions further confirming the significance of nucleosides/nucleotides for placental growth and pregnancy outcomes. We showed that the term placenta and placental experimental models express adenosine receptors, nucleoside transporters, and enzymes involved in adenosine metabolism. Adenosine did not affect the differentiation and syncytialization of cytotrophoblast and apoptosis of the proliferating cytotrophoblast and regulated adenosine receptors, nucleoside transporters, and adenosine metabolism. Adenosine or other nucleosides accelerated cytotrophoblast proliferation. Cornerstone of ART of HIV in pregnancy, purine analog abacavir known to be transported by nucleoside transporters did not show any effects, but in supratherapeutic concentration, it increased adenosine receptor A2B in BeWo cells. Overall, our study emphasizes the importance of nucleosides for cytotrophoblast proliferation and the regulatory effects of adenosine in the placenta. Abacavir at therapeutic concentrations seems not affect adenosine metabolism, transport, and signaling. Thus it can be concluded that in these terms it is safe pharmacotherapy.

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BPT 9

ROLE OF CXCL14 IN AML AND CML

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Chemokine ligand 14 (CXCL14) is a small cytokine belonging to the CXC chemokine family playing an important role in immune system. CXCL14 is often dysregulated in several types of carcinomas and some studies have indicated that restoration of CXCL14 expression in cancer cells supresses tumor growth. To our knowledge, there is no study discussing the effect of CXCL14 on acute myeloid leukemia (AML) cells in the bone marrow (BM) microenvironment. However, the recent study suggests CXCL14 as a new therapeutic option in chronic myeloid leukemia (CML)[1]. The aim of our present work was to verify the effect of CXCL14 in CML cell lines and to address the possible role of CXCL14 in AML cells. Using NIH/3T3 stromal CML cells or isolated CML patient stromal cells in co-cultures with K562 cells we confirmed that CXCL14 synergizes with the antiproliferative effect of imatinib. We verified these results using the CFU-C assay, where the CML cells stimulated with CXCL14 and imatinib formed the least colonies. In AML cells we observed that the exposure to CXCL14 for 3 days promotes cell proliferation, while longer 5 days exposition significantly decreased the cell count of AML cell line MV4-11 and THP-1. In addition, after 7 days exposition of MV4-11 AML cells to CXCL14 in combination with azacytidine, significantly decreased cell count compared to the treatment of cells with azacytidine alone. Our data thereby confirm the role of CXCL14 in CML cells and suggest that similar effect on proliferation, maturing and drug resistance can be observed in AML cells as well.

The study was supported by the Czech Health Research Council (AZV 170/52/235201, AZV 170/60/2360521.)

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BPT 10

EFFICACY OF SELECTED TYROSINE KINASE INHIBITORS IN REVERSING PHARMACOKINETIC MULTIDRUG RESISTANCE: AN *EX VIVO* AND *IN VIVO* STUDY

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The multidrug resistance (MDR) poses a significant obstacle in achieving successful outcomes of cancer chemotherapy. This phenomenon is observed in various tumor types, including non-small cell lung cancer (NSCLC). ATP-binding cassette (ABC) drug efflux transporters are considered to be a critical factor involved in the development of MDR. In our current study, we have evaluated MDR-combating abilities of two novel kinase inhibitors (TKIs) approved for NSCLC therapy in *ex vivo* and *in vivo* human tumor models. First, a microarray assay and next-generation sequencing were used to determine ABC transporter expression levels and target mutational statuses, respectively, in primary three-dimensional (3D) tumoroids derived from NSCLC biopsies. Subsequently, tested TKIs were examined for their possible ability to synergize with topotecan in this *ex vivo* model. As a result, a potent MDR-modulatory capacities of selected inhibitors were recorded in 3D tumoroids positive for ABC transporters' expression. Currently, the observed MDR-reversal effect is being verified in *in vivo* drug combination assays with patient-derived xenograft (PDX) mouse models. Our study will reveal whether the selected TKIs might be clinically relevant MDR chemosensitisers in combination NSCLC chemoregimes.

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BPT 11

ENDOGLIN BLOCKAGE IS CRUCIAL IN INFLAMMATION-INDUCED ENDOTHELIAL DYSFUNCTION IN VITRO

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Endoglin (Eng), the TGF-β co-receptor, was previously shown to play an important role in endothelial dysfunction, the first step of atherosclerosis. TRC105 is an antibody that can block Eng and its signaling. Our aim was to investigate the TRC105 effects on the Eng expression and function in inflammation-induced endothelial dysfunction. Human aortic endothelial cells (HAECs), passage 5, were treated with TNF-α (10 ng/mL) for 4 hours, followed by the addition of TRC105 (300 μg/mL) for 12 hours. Protein levels, adhesion, and transmigration of monocytes were assessed by flow cytometry, mRNA expression was measured by qRT-PCR, and levels of soluble Eng were measured by ELISA. Inflammation induced by TNF-α resulted in decreased protein expression of Eng and increased protein expression of cell adhesion molecules (ICAM-1, VCAM-1). This was followed by increased adhesion of THP-1 monocytes to endothelial cells, suggesting that the effect of adhesion molecules prevails over the Eng effect. However, treatment with TRC105 led to further reduction of Eng protein expression, which was able to prevent inflammation-induced adhesion of monocytes to endothelial cells. These results show that blockage of Eng by TRC105 can prevent the endothelial dysfunction induced by TNF-α in HAECs, suggesting that Eng participates in inflammation-induced endothelial dysfunction, but to which extent must be further investigated.

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BPT 12

BLOCKAGE OF ENDOGLIN PREVENTS ENDOTHELIAL DYSFUNCTION DEVELOPMENT IN TYPE 2 DIABETIC CORONARY ARTERY ENDOTHELIAL CELLS

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Endoglin (Eng) is a cell membrane glycoprotein expressed in the vascular endothelium, which is related to endothelial dysfunction and inflammation. TRC105 (carotuximab) is a monoclonal antibody that binds to membrane Eng, and it was published that direct blockage of Eng can potentially prevent hypercholesterolemia- or hyperglycemia-induced development of endothelial dysfunction. Therefore, we hypothesized that TRC105 prevents endothelial dysfunction development in coronary artery endothelial cells from type 2 diabetes mellitus patients. We aimed to evaluate the impact of TRC105 treatment on Eng expression and function with respect to endothelial dysfunction and inflammation by comparing human coronary artery endothelial cells from healthy donors (HCAEC) with human coronary artery endothelial cells from type 2 diabetes mellitus patients (D-HCAEC). Cells were cultured in EGM-2 media with appropriate supplements and 10% FBS and were treated with TRC105 (300μg/ml) for 12 hours. For protein analysis, samples were analyzed by flow cytometry. For functional analysis, adherent and transmigrated THP-1 monocytes to endothelial cells were quantified by flow cytometry. The protein analysis showed that Eng, inflammatory markers such as 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. TRC105 treatment significantly reduced Eng expression, which resulted in decreased adhesion of monocytes to endothelium. These results suggest the crucial role of Eng in endothelial dysfunction development in endothelial cells from type 2 diabetes mellitus patients.

The study was supported by GAUK 288322 and SVV 260663.

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BPT 13

MONOAMINE HOMEOSTASIS IN THE FETOPLACENTAL UNIT; EFFECT OF TROPHOBLAST DIFFERENTIATION AND PHARMACOTHERAPY

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In pregnancy, the placenta is of great importance for proper fetal growth and development. Its crucial function is the homeostasis of biogenic amines in the fetoplacental unit, such as serotonin (5-HT), norepinephrine (NE), and dopamine (DA). Responsible are the monoamine transporters located in the differentiated syncytiotrophoblast (STB), and its progenitor cells, the cytotrophoblasts (CTBs); namely, serotonin transporter (SERT), norepinephrine transporter (NET), and organic transporter 3 (OCT3). Unfortunately, knowledge of trophoblast monoamine system regulation and functional characteristics is sparse. Similarly, its status in the undifferentiated CTB cells remains unclarified. Furthermore, it has been ascertained that maternal factors, such as pharmacotherapy (e.g. antidepressants and antidiabetics), can seriously impact trophoblast function. Therefore, in this work, we used a range of human- and rat-derived experimental approaches to investigate the expression and functionality of monoamine transporters involved in the trophoblast monoamine homeostasis, and the effects of pharmacotherapy herein. The outcomes of this work expand the knowledge of monoamine placental handling and drug safety in pregnancy.

The study was supported by the Czech Science Foundation (GACR 20-13017S), the Grant Agency of Charles University (GAUK 358821/C/2021, SVV 260414), and the project National Institute for Neurological Research (Programme EXCELES, ID Project No. LX22NPO5107) - Funded by the European Union - Next Generation EU.

BPT 14

ENHANCED FERRITIN NANOPARTICLES FOR ENCAPSULATION AND TARGETED DELIVERY OF DRUGS

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Ferritin, a naturally occurring iron storage protein, is a promising drug delivery platform thanks to its inherent biocompatibility and capacity to encapsulate therapeutic agents. In this study, we prepared genetically engineered human H ferritin incorporating 4 or 6 tryptophan residues per subunit, strategically orientated toward the inner cavity of the nanoparticle to enhance its ability to encapsulate hydrophobic drugs in the ferritin cage. Comprehensive characterisation of the mutants revealed that only the variant that carried four tryptophan substitutions per subunit retained the ability to disassemble and reassemble properly. As a proof of concept, we evaluated the loading capacity as well as cellular uptake and cytotoxic effects of these nanoparticles loaded with ellipticine, a natural hydrophobic indole alkaloid with multimodal anticancer activity, or doxorubicin. Our data demonstrated that this specific mutant exhibited significantly higher loading efficiency compared to human H ferritin as well as efficient uptake by promyelocytic leukemia derived cells resulting in the expected cytotoxic effect.

The results of our study highlight the potential of HFt-W4 as a robust drug delivery system, especially for more hydrophobic molecules, that can target cancer cells through TfR1 receptor.

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CHANGES IN THE EXPRESSION AND ACTIVITIES OF DETOXIFICATION ENZYMES IN THE IN VIVO MODEL OF NAFLD

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Non-alcoholic fatty liver disease (NAFLD) is a chronic liver disease with a spectrum of liver abnormalities, typically commencing with benign simple steatosis. In a subset of patients, this condition may progress to a more severe form known as non-alcoholic steatohepatitis (NASH), either with or without subsequent advancement to liver fibrosis, which carries the potential to escalate into life-threatening conditions such as liver cirrhosis and hepatocellular carcinoma. Given that NAFLD impacts about 25% of the global population, and its prevalence is on the rise in tandem with the escalating rates of obesity, metabolic syndrome, and type 2 diabetes mellitus, the objective of this research was to investigate the development of NAFLD and its effects on detoxification enzymes and epigenetic regulators in NMRI mice. This investigation involved both control NMRI mice and NMRI mice treated with monosodium glutamate (MSG), all of which were subjected to either a standard diet (STD) or a high-fat, high-fructose, and high-cholesterol (FFC) diet. We observed pronounced liver steatosis and a significant increase in the body weight and liver weight of both control and MSG mice fed a FFC diet compared to their respective control groups at the age of 7 months. Following the administration of a FFC diet or MSG, the most notable alterations in both the expression and activities of detoxification enzymes were observed in glutathione peroxidase (GPx), glutathione S-transferase (GST), cytochrome P450 1A1/2 (CYP1A1/2), aldo-keto reductase 1C (AKR1C), carbonyl reductase 1 (CBR1), and NAD(P)H:quinone oxidoreductase 1 (NQO1). Furthermore, the gene expression of selected microRNAs and genes associated with epigenetic regulation was predominantly altered in the liver of MSG STD mice.

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BPT 16

TRANSGENIC MICE OVEREXPRESSING HUMAN ENDOGLIN AS A PROPER ANIMAL MODEL TO STUDY THE IMPACT OF ENDOGLIN ON ENDOTHELIAL DYSFUNCTION AND LIVER DISORDERS

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Endoglin (CD105, ENG) is a transmembrane glycoprotein and a coreceptor of the Transforming Growth Factor β (TGFβ) superfamily. ENG expression is changed during aortic endothelial dysfunction, liver fibrogenesis, and sinusoidal endothelial dysfunction. Furthermore, blocking of ENG expression and signaling prevented cholesterol and glucose-induced adhesion and transmigration of monocytes through endothelial monolayers. However, ENG overexpression has never been studied in relation to the development of endothelial dysfunction and liver alterations. Therefore, the goal of this study was to develop such an animal model for studying the essential role of ENG in endothelial dysfunction and liver disorders. To describe mouse and human ENG expression in the aorta and liver, transgenic mice expressing human L-endoglin (L-ENG+) on CBAxC57BL6J background were generated. ENG expression was investigated in the liver and aorta of L-ENG+ mice and their WT littermates, with a focus on human and mouse ENG expression and location. Western blot analysis confirmed the presence of human ENG in the aorta and liver of L-ENG+ mice, but not in their WT littermates. However, mouse ENG expression in the aorta and liver of WT and L-ENG+ did not differ significantly. In immunohistochemical analysis, human ENG was found only in liver sinusoidal endothelial cells of transgenic mice, whereas mouse ENG was found in liver sinusoidal endothelial cells of both transgenic L-ENG+ and WT mice. These findings demonstrate that the transgenic L-ENG+ mouse model will be beneficial in studying the role of ENG in the development of aortic endothelial dysfunction during atherogenesis and liver sinusoidal endothelial dysfunction in liver disorders.

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RADIOLABELED 15-MER PEPTIDES ARE MEGALIN LIGANDS IN CRISPR/CAS9-BASED CELLULAR MODEL

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Megalin (LRP2 receptor) mediates endocytosis of high molecular weight compounds[1] including radiolabeled peptides. Internalization of radiolabeled peptides in proximal tubular cells may lead to nephrotoxicity due to accumulation of radioactive tracer.[2]

HK2 cells naturally expressing LRP2 were genetically modified using CRISPR/Cas9 technique for accumulation studies of radiolabeled 15-mers, the peptides designed to target VEGF (Vascular Endothelial Growth Factor) receptors in oncology diagnostic. The *LRP2* edits were verified by flow cytometry analysis, viability assays and accumulation studies. The FITC-albumin accumulation mediated by megalin was employed in the FACS (Fluorescence-Activated Cell Sorting) method to separate modified *LRP2* KO subclones. The sorted cells were used for accumulation assays with radiolabeled [^{68}Ga]Ga-NODAGA-15-mer and [^{99m}Tc]Tc-KDC-15-mer. The radiolabeled 15-mer was confirmed as a megalin ligand irrespective of the way of radiolabeling.

The *LRP2* KO human kidney HK2 cells thus represent a suitable model for the preclinical testing of radiolabeled peptides.

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A NEW WAY TO SIMULATE INTERNAL INTRINSICALLY DISORDERED PROTEINS: AN MD INVESTIGATION INTO P53

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Unraveling the mysteries of Intrinsically Disordered Proteins (IDPs) and Regions (IDRs) is one of the greatest challenges in the 21st century. The flexibility of these regions allow proteins to adopt vastly different conformations, allowing them to facilitate the binding and unbinding of vital activation sites. These regions, even in well-studied proteins such as the tumor suppressor p53 protein, are not well understood. We utilized data from a CryoEM structure of p53, and terminally locked the regions to reflect the existence of neighboring globular regions on the behavior. We ran trajectories with the end-to-end distances locked at 0.5, 3.0, 5.0 and 7.0 nm to reflect to observe the changes in the types and quantities of secondary structures. We computed the average chemical shifts (CS) for each of these trajectories and found that experimental[1] CS agreement peaks at the distance specified in the CryoEM structure,[2] with root-mean-squared deviation improved by between 0.12 and 0.26 ppm (depending on the atom) from the traditional MD trajectory (unrestricted). The results of this investigation are to be published in JCTC entitled “Exploring the Role of Globular Domain Locations on an Intrinsically Disordered Region of p53: A Molecular Dynamics Investigation” along with several other articles in the works about other regions within p53. The goal for this investigation is to give a complete understanding of the multiple conformations of p53, provide insight into the performance of flexible IDRs in primarily globular proteins, and pave the way for a collaborative SAXS investigation into the inner machinations of these elusive structures.

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BPT 19

EVALUATION OF NOVEL FLT3 INHIBITORS IN ACUTE MYELOID LEUKEMIA

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Acute myeloid leukemia (AML) is characterized by the excessive proliferation of myeloid progenitor cells to immature myeloblasts and poor overall survival of the patients. Approximately 30 % of AML patients have FMS+-like tyrosine kinase 3 (Flt3) mutation. Recently, Flt3 inhibitor gilteritinib has become an important part of the pharmacotherapy for Flt3-positive patients. Nevertheless, several mechanisms have been described to mediate resistance to the drug. Therefore, intensive effort is being put into finding new potent and specific Flt3 inhibitors that can overcome resistance to gilteritinib-containing therapy.

Here we aimed to evaluate 30 newly synthesized investigative compounds as potential Flt3 inhibitors. MTT assay on AML cell lines MOLM-13, MV4-11, and THP-1 revealed eight of this first set of structures inhibiting the cells proliferation, with higher effect in the Flt3 mutation carrying MOLM-13 and MV4-11 than in the Flt3 non-mutated ones. Subsequently, 13 new derivates of DS-28, the most potent substance of the first set, were tested as described above. New derivate LG-2140 revealed even higher efficacy (IC_{50} = 0,1603 μM for MV4-11 and 0,2435 μM for MOLM-13) than DS-28 and also better selectivity to Flt3 receptor.

Newly generated MV4-11 g45 cells with permanent resistance to gilteritinib (RF = 3,335) will be used to address the ability of our investigative compounds to overcome resistance to the Flt3 inhibitor. Further experiments will be needed employing AML patients´-derived mononuclear cells to reveal possible therapeutic value of the most effective lead compounds.

The study was supported by the Czech Health Research Council (AZV 23-08-00439 and AZV 23-03-00562)

BPT 20

EFFECT OF THE AURORA A TYROSINE KINASE INHIBITOR ALISERTIB ON ANTHRACYCLINE RESISTANCE IN CANCER CELLS

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Cancer treatment resistance presents a significant challenge in the field of clinical oncology. Aurora kinases (AURK) have emerged as a promising target for cancer treatment, particularly in Acute Myeloid Leukemia (AML). Clinical trials have highlighted the importance of AURK inhibitors in various contexts, leading us to investigate their efficacy in both alone and in combination with anthracyclines (ANT), which are known as standard chemotherapeutic regimens. These trials are crucial due to the high effectiveness of anthracyclines, which is hindered by the potential development of resistance mechanisms. One such mechanism is metabolic inactivation, driven by carbonyl-reducing enzymes (CREs) like Aldo-Keto Reductases (AKRs), which poses a significant challenge. AKRs-mediated resistance, induced by exposure to ANTs, involves a complex interplay within cancer cells, as they catalyze the reduction of anthracyclines to secondary alcohols, thereby reducing the effectiveness of ANT in the process of treatment. Alisertib (ALI), a selective inhibitor of Aurora kinase A (AURKA), is currently being investigated in clinical trials for the treatment of various malignancies, including AML, and has recently been approved for small lung cancer treatment. Our project builds upon compelling preliminary data that suggests ALI's ability to overcome AKR-mediated drug resistance. We aim to determine the impact of ALI on the reduction of daunorubicin (DAUN) catalyzed by AKR1C3. Initially, we conducted the incubation methods using the recombinant AKR1C3 enzyme. Subsequently, we performed inhibitory studies and drug combination assays using cell lines. The results obtained demonstrate the ability of ALI to inhibit both recombinant AKR1C3 and endogenous AKR1C3. In conclusion, ALI effectively modulates AKR1C3 activity, indicating that combining ALI with DAUN could be a promising approach to counter ANT resistance in cancer.

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BPT 21

NEW MECHANISM OF ANTINEOPLASTIC ACTIVITY OF ZANUBRUTINIB VIA INHIBITION OF AKR1B10

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Aldose reductase-like 1 protein AKR1B10 is a member of the Aldo-keto reductase family overexpressed in several malignancies such as leukemia, hepatic, lung, breast, cervical, kidney, pancreatic, and endometrial cancers, where it reduces α- and β-unsaturated carbonyls protecting host cell from carbonyl toxicity. Also, AKR1B10 may promote carcinogenesis via retinal, farnesal, and geranylgeranial reduction. It has restricted expression in healthy tissues, primarily the small intestine and colon, thus providing a selective target for cancer treatment. Here is presented the inhibition of AKR1B10 by second generation Bruton's tyrosine kinase inhibitor zanubrutinib, a drug already approved for treating particular lymphomas and chronic lymphocytic leukemia. Zanubrutinib inhibits AKR1B10 metabolism of daunorubicin *in vitro* with an IC_{50} value of 8 μM, suggesting it may be used as a promising off-label treatment in many other cancers, alone or in combination with anthracyclines. The possible binding mode and structure-activity relationship were examined via molecular docking.

QUANTITATIVE ANALYSIS OF GLUCOSE-RELATED GENES ACTIVATED BY RIFAMPICIN

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Certain studies suggest that rifampicin may have a hyperglycemic effect and can cause diabetes[1,2]. The reasons to encounter this side effect can be due to microbial inflammation or as a result of treatment[3]. In this presentation, we will focus specifically on the effect of rifampicin as a treatment for tuberculosis patients. The literature mentioned that the nuclear receptor PXR, which is activated by rifampicin plays a crucial role in glucose metabolism[4]. For instance, this nuclear receptor induces gluconeogenesis genes, such as G6PC and PCK1, but inhibits glucose uptake transports, like GLUT2 [5]. The mechanism of these genes is described quantitively by novel mathematical time-dependent models. These models allow us to predict glucose-related gene transcription after treatment with rifampicin and at different initial doses. Moreover, the analysis of model solutions can serve to predict the hyperglycemic phase after the treatment of patients. Finally, a pre-conclusion is drawn for the impact of rifampicin on glucose homeostasis.

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MATHEMATICAL MODELING OF RIFAMPICIN METABOLISM IN PRIMARY HUMAN HEPATOCYTE SPHEROIDS

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Rifampicin (RIF), a potent pregnane x receptor (PXR) agonist, upregulates hepatic enzymes, including cytochrome P450 3A4 (CYP3A4), impacting drug metabolism[1]. RIF is extensively metabolized in the liver to its active metabolite, 25-desacetylrifampicin (DRIF), by arylacetamide deacetylase (AADAC)[2]. However, current computational models[3] neglect the role of this metabolite. To address this gap, we develop a mechanistic model (MM) to examine the metabolism of RIF by AADAC and the consequent effects on PXR activation and CYP3A4 expression within 3D primary human hepatocyte spheroids (PHHs). The goal is to ascertain the conversion rate of RIF to DRIF, delineate each molecule's role in PXR activation, and analyze the resulting CYP3A4 expression kinetics at varying RIF metabolism rates. Initial results from our MM, calibrated with CYP3A4 mRNA fold change data from 3D PHHs, show that both RIF and DRIF, activate PXR, and CYP3A4 expression is influenced by RIF's metabolic rate. Ongoing efforts are focused on refining the MM through sensitivity analysis of RIF's metabolism rates and further experimental validation to confirm these findings. Integrating experimental data with mathematical modeling promises to enhance our understanding of RIF metabolism, with potential applications to other drugs and metabolic pathways.

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CHARACTERIZATION OF NOVEL CAR LIGANDS IN HEPATIC CELLS

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The constitutive androstane receptor (CAR, NR1I3) belongs to the nuclear receptor family. Like other nuclear receptors, it is involved in the regulation of the metabolism of exogenous and endogenous substances in the liver.[1] Recent studies have shown that CAR ligands significantly regulate lipids, glucose, and bile acid metabolism. These data were obtained in mouse models with the murine CAR ligand TCPOBOP.[2] Unfortunately, there is currently no human ligand to help understand the function of the human CAR receptor and demonstrate its use as a molecular target in pharmacotherapy.

In the past, our group has been involved in the discovery of several novel human CAR receptor ligands. Following our previous discovery, a series of fluorinated imidazo[1,2-a]pyridine compounds was prepared to examine their ability to activate the human CAR receptor selectively. In this study, we ran a series of cellular experiments such as TR-FRET CAR assay, PXR, LXR, FXR, CAR assembly, and CAR3 assay to examine the CAR receptor activity and nuclear receptor specificity of our ligands. We found that four out of six compounds possess significant activity to the CAR receptor in hepatic cells. Noteworthy is the ligand MI763, which was able to activate human CAR receptor at nanomolar concentrations. This compound may serve as a potential candidate for cholestasis treatment; therefore, experiments in animal cholestasis models will be accomplished in future research.

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BPT 25

RHO-ASSOCIATED PROTEIN KINASE 1 INHIBITION IN HEPATOCYTES ATTENUATES NONALCOHOLIC STEATOHEPATITISRESENTATION

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NASH is the progressive form of NAFLD characterized by lipotoxicity, hepatocyte injury, tissue inflammation, and fibrosis. Previously, Rho-associated protein kinase (ROCK) 1 has been implicated in lipotoxic signaling in hepatocytes in vitro and high-fat diet-induced lipogenesis in vivo. However, whether ROCK1 plays a role in liver inflammation and fibrosis during NASH is unclear. Here, we hypothesized that pathogenic activation of ROCK1 promotes murine NASH pathogenesis.

Patients with NASH had increased hepatic ROCK1 expression compared with patients with fatty liver. Similarly, hepatic ROCK1 levels and activity were increased in mice with NASH induced by a western-like diet that is high in fat, fructose, and cholesterol (FFC). Hepatocyte-specific ROCK1 knockout mice on the FFC diet displayed a decrease in liver steatosis, hepatic cell death, liver inflammation, and fibrosis compared with littermate FFC-fed controls. To explore the therapeutic opportunities, mice with established NASH received ROCKi, a novel inhibitor of ROCK1/2, which preferentially accumulates in liver tissue. ROCK inhibitor treatment ameliorated insulin resistance and decreased liver injury, inflammation, and fibrosis.

Genetic or pharmacologic inhibition of ROCK1 activity attenuates murine NASH, suggesting that ROCK1 may be a therapeutic target for treating human NASH.

BPT 26

MODULATION OF ENDOGLIN AND SOLUBLE ENDOGLIN IN NASH: EXPLORING THE THERAPEUTIC BENEFITS OF MONOCLONAL ANTIBODY

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NASH treatment requires a comprehensive understanding of its molecular progression. Endoglin, a TGFβ superfamily coreceptor, exist in membrane (ENG) and soluble (sENG) forms. While ENG is linked to liver sinusoidal endothelial dysfunction (LSED) and liver fibrosis, its role in NASH remains unclear. We aimed to explore the link between NASH, ENG, and LSED. We hypothesized anti-endoglin antibody M1043 would affect LSED by directly affecting ENG. NASH was induced in two male C57BL/6 mice groups with CDAA-HFD diet. Mice were sacrificed after 4 and 8 weeks to assess ENG changes. Then, 24 mice were divided into control, CD+rat IgG, and CD+M1043 groups. After 4 weeks, CD+IgG and CD+M1043 mice received i.p. injections of rat IgG (10mg/kg) and M1043 (10mg/kg) twice a week and the experiment lasted 8 weeks. A computational model of ENG modulation of TGFβ signaling was developed to understand the role of ENG antibody and its cleavage. Elevated liver enzymes, fibrosis, and inflammation confirmed liver injury. LSED was proven by risen ENG, VCAM-1, and ICAM-1 expression. Increased MMP-14 and sENG levels in 8 weeks mice was shown. M1043 effectively prevented the increase in ENG, VCAM-1, and ICAM-1. Computational modeling simulations showed an ENG-dependent switch in signaling and sENG levels, reversed by ENG antibodies. Our findings suggest an association between NASH, LSED and ENG. This implies a potential role of ENG in LSED. Blocking ENG might be a possible target to affect LSED and probably prevent NASH progression.

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BRUTON'S TYROSINE KINASE INHIBITOR, EVOBRUTINIB, AS A NOVEL INHIBITOR OF ALDO-KETO REDUCTASE 1C3 IN THE FIGHT AGAINST RESISTANCE IN DAUNORUBICIN-BASED CANCER THERAPY

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In the field of cancer therapy, multidrug resistance is defined as the ability of cancer cells to survive treatment with a variety of anticancer drugs. There are many mechanisms involved in multidrug resistance. Numerous studies have implicated human aldo-keto reductases (AKRs) in drug resistance to anthracyclines (daunorubicin and doxorubicin). AKRs are overexpressed in a large number of human tumors and mediate resistance to cancer chemotherapeutics by being directly involved in their metabolism. In most cases, they catalyze the reduction of carbonyl groups to alcohols. Aldehydes are converted to primary alcohols and ketones are converted to secondary alcohols on a variety of substrates. In recent years, several research groups have described inhibitors to restore sensitivity to anthracycline drugs[1,2].

In this regard, this study investigates the pharmacokinetic drug-drug interactions of evobrutinib with daunorubicin and AKRs. The inhibition of the reductive conversion of daunorubicin to its metabolite daunorubicinol and the potential effect of the combination of daunorubicin with the appropriate AKR inhibitor was investigated. We demonstrated that evobrutinib efficiently prevents AKR1C3-mediated inactivation of daunorubicin, both in its recombinant form as well as during its overexpression in cancer cells.

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