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# Abstracts

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**BPT1**

# ASSESSMENT OF DNA DAMAGE INDUCED BY EPIRUBICIN IN ISOLATED ADULT RAT CARDIOMYOCYTES

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Anthracycline antibiotics (ANT, e.g. doxorubicin, epirubicin) are antineoplastic drugs that have been in clinical use for more than 60 years. Their mechanism of action is complex and dose-dependent. ANTs inhibit both isoforms of topoisomerase two, intercalate into DNA and form DNA adducts that lead to DNA damage. These effects target both cancerous and non-dividing cells. In addition to antineoplastic effects, ANTs cause acute and long-term toxicities such as cardiotoxicity and gonadotoxicity. Existing cell models for cardiotoxicity studies, such as the H9c2 line and neonatal rat cardiomyocytes, have limitations in reproducing the adult cardiac phenotype. The commonly used neonatal rat cardiomyocyte model is a good way to study the long-term effects of anthracyclines because it is stable in culture for several days. However, because heart cells are isolated from newborn rats, protein expression is different from adult heart tissue. This study presents a method to isolate adult rat cardiomyocytes using collagenase perfusion via the Langendorff system. Isolated cells were plated on laminin-coated plates and treated with epirubicin. Alkaline comet assay showed that epirubicin caused DNA damage in isolated adult heart cells. This approach provides another physiologically relevant system to study mechanisms of anthracycline-induced cardiotoxicity and potentially for the development of new cardioprotective agents.

**BPT 2**

# CONSTITUTIVE ANDROSTANE RECEPTOR ACTIVATION DECREASES LIVER CONTENT OF BILE ACIDS DURING ESTROGEN-INDUCED CHOLESTASIS IN MICE

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Bile acids (BAs) are steroid compounds secreted primarily by bile with numerous endocrine and exocrine functions. BAs accumulate during cholestasis and contribute to liver damage. The constitutive androstane receptor (CAR) is a nuclear receptor involved in the regulation of BA homeostasis, but the effect of its activation during different forms of cholestasis has not been comprehensively studied.[1] In the present study, we analyzed the changes in BA homeostasis induced by CAR activation in mice with estrogen-induced (EE) cholestasis, a model mimicking intrahepatic cholestasis in pregnancy (ICP). Mice received ethinylestradiol, and CAR was stimulated by TCPOBOP. TCPOBOP restored EE-induced impairment of bile flow by increasing biliary secretion of glutathione and BA. These activations were possible due to the regulation of Slc10a1 (Ntcp) and Abcc2 (Mrp2) transporters and Cyp7a1 and Cyp3a11 mRNA, which are involved in BA homeostasis in the liver. Next, we developed a novel potent human CAR ligand from a recently published library. Initial studies in humanized hPXR/hCAR/hCYP3A mice suggest that the compound MI763F regulates the same genes as murine ligand TCPOBOP in the liver. In conclusion, our results suggest that CAR receptor activation may protect against EE-induced cholestatic liver injury by attenuating BA accumulation in the liver.

*The study was supported by GAUK No. 170/50/245002.*

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**BPT 3**

# TAPEWORMS AS AN IMPORTANT PLAYER IN TRANSPORT AND METABOLISM IN THE GUT

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Tapeworms (Cestoda) are common intestinal helminthic parasites of all vertebrates including humans. Significantly modulating the gut environment by releasing a cocktail of excretion-secretion products, the tapeworms could bring both beneficial and detrimental effects to its hosts: Besides the immunomodulating potential leading to alleviation of bowel pathologies, nonphysiological state of the gut together with tapeworm uptake and metabolism decrease the availability of certain chemicals for the host. This effect could be counterintuitively beneficial, as the tapeworms reduce the exposure of the host to e.g. organic and inorganic pollutants. On the other hand, tapeworms could play negative role in pharmacokinetics by metabolizing or uptaking the drugs and thus denying the host a sufficient drugs concentration. Protective potential from oxidative stress caused by inorganic metalloids (Aresenic, specifically) as well as its ability to uptake and metabolize anthelmintics such as monepantel or levamisole has not yet been described in tapeworms and is subject of our current research. We aim to investigate this phenomenon using a model tapeworm *Hymenolepis diminuta* and combinig *in vitro* and *ex vivo* methodologies using activity measurement of chosen xenobiotics-metabolizing (e.g. Glutathione S-transferase) and antioxidant enzymes (e.g. Catalase) in combination with monitoring of parental drugs and their metabolites as well as oxidation states of arsenic.

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# EFFICACY OF NEW BENZHYROXAMIC ACID DERIVATES ON NEMATODE

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Parasitic nematodes are responsible for a wide range of diseases in animals, including humans. However, the efficacy of existing anthelmintic drugs, which are commonly used to treat these infections, is waning due to the increasing prevalence of drug resistance in nematode populations. This growing challenge underscores the urgent need to discover and develop novel nematocidal drugs[1]. In the present study, 13 novel derivatives of benzhydroxamic acid (OMKs) were designed and synthesized, and their anthelmintic activity was tested in the nematodes *Haemonchus contortus* and *Caenorhabditis elegans* as a model organism, with classical anthelmintics (levamisole etc.) used for comparison.

Among the derivatives, OMK211 demonstrated the most favourable outcomes, exhibiting a reduction in viability and motility of larval and adult stages of both drug-sensitive and drug-resistant strains of *H. contortus*. Additionally, OMK211 was found to be non-toxic to mammalian cells in vitro and in vivo. Subsequently, thermal proteome profiling analysis was employed to ascertain the molecular target of OMK211 in *H. contortus.* In conclusion, these findings suggest that novel derivatives of benzhydroxamic acid represent a promising new class of potential anthelmintics with a novel mechanism of action.

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**BPT 5**

# THE ROLE OF HISTONE MODIFICATIONS IN THE DEVELOPMENT OF DRUG RESISTANCE IN THE PARASITIC NEMATODE *H. CONTORTUS*

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The ever-growing drug resistance in *H. contortus* poses challenges for livestock farming and associated economic losses, and risks of transmission to wildlife like deer and mouflon. Addressing this resistance requires multiple strategies, including developing new drugs targeting known molecular sites, identifying new targets, and exploring drugs outside of the anthelmintic category. Histone modification represents one approach to investigate drug resistance and its transmission to the next generation in the form of so-called transgenerational epigenetic inheritance, which has been previously described in both mice and the model nematode *C. elegans* [1]. This epigenetic mechanism alters gene expression by modifying DNA without changing its structure, mainly through N-acetylation and N-methylation of lysine or arginine residues, which affects chromosome condensation and gene transcription of certain genome regions. These modifications are mostly carried out by enzymes of the histone acetyltransferase (HAT) and histone deacetylase (HDAC) family. This project aims to determine whether changes in histone modification might be related to the development of drug resistance in the parasitic nematode *H. contortus*. A bioinformatic *in silico* analysis was performed to select homologous domains of HAT and HDAC compared to *C. elegans*. In addition, transcriptional analysis of selected HAT and HDAC was performed for all developmental stages of *H. contortus* (eggs, larvae, adults), comparing both the susceptible strain (ISE) and the resistant strain (IRE). All developmental stages were exposed to sublethal doses of albendazole, and the expression of the same genes was also determined.

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**BPT 6**

# THE EFFECT OF FLUBENDAZOLE ON PANCREATIC CANCER CELL LINES

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Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive malignancy with the worst prognosis among all cancers, as only 5–10% of patients survive beyond five years post-diagnosis. Improving treatment effectiveness remains a key priority in current research.[1] As flubendazole (FLU), a benzimidazole anthelmintic, has previously demonstrated antitumor activity by inhibiting microtubule polymerization in several type of cancers[2], this study aimed to evaluate the therapeutic potential of FLU on PDAC cells and explore its role in identifying novel treatment strategies for PDAC.

Stabilized PDAC cell lines (BxPC-3, PANC-1, MIA-PaCa-2, Patu-8902) were used to assess FLU's efficacy. Cell viability was determined using the WST-1 assay for 2D cultures and the CellTiter-Glo® 3D assay for 3D cultures derived from the BxPC-3 line and prepared with Geltrex. Morphological changes were observed using phase-contrast and fluorescence microscopy. Significant differences in gene and protein expression were identified across the tested cell lines via qPCR and western blotting.

Our findings demonstrate that FLU exerts potent antitumor effects on PDAC cell lines by inhibiting microtubules, highlighting its potential as a promising therapeutic candidate for further investigation in both, 2D and 3D cancer models.

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**BPT 7**

# RIBOSOME-INACTIVATING PROTEINS AS CARGO IN PHOTOCHEMICAL INTERNALIZATION VIA PHTHALOCYANINE PHOTOSENSITIZERS

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Photochemical internalization (PCI) is one of the applications of photodynamic therapy (PDT) in the experimental or clinical field. The common features of these two methods is the use of three components: light, oxygen and a photosensitizer (PS). The disadvantage of traditional methods of tumor treatment is their invasiveness and interference with healthy tissues. In contrast, the PCI and PDT are characterized by low toxicity to untreated tissue thanks to localized irradation to treated area. The individual elements used in this methodology are non-toxic on their own, but their combination results in a photodynamic and photochemical effects with the formation of reactive oxygen species (ROS).[1] Unlike PDT, photodynamic effect and production of ROS is not the mechanism of action in PCI. In PCI, PS is merely used in low doses to release active compounds from endolysosomal compartment to cytosol, and thus prevent their degradation in lysosomes. This allows better penetration of the active substance into the target tissue and thus improve the effectiveness of the treatment.[2]
In this work, we are comparing original amphiphilic phthalocyanines with commercially available PSs ($AlPcS_{2a}$ and $TPPS_{2a}$)[3] on the HeLa cell line (cervical carcinoma) in combination with saporin or gelonin (ribosome-inactivating proteins; RIPs). Two approaches were employed: “light-after” (co-incubation of PS with RIPs and subsequent irradiation) and “light-before” (RIPs are applied to the cells after the PDT treatment). Combination studies demonstrated synergistic effect. Our original PSs showed comparable or stronger effect than PSs traditionally used in PCI research ($AlPcS_{2a}$ and $TPPS_{2a}$).

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**BPT 8**

# ELIMINATION OF PD-L1+ LUNG CANCER CELLS USING PHOTOCHEMICAL INTERNALIZATION

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Photochemical internalization (PCI) is an innovative technique designed to enhance intracellular drug delivery by releasing endocytosed macromolecules into the cytoplasm. This process utilizes photosensitizers localized in the membrane of endosomes that, upon light activation, disrupt the vesicle membranes, enabling the release of therapeutic agents into the cytoplasm. PCI has demonstrated the potential to enhance the efficacy of several macromolecular therapeutics, including chemotherapeutics, gene therapy agents, and cancer vaccines. Moreover, it has shown promising results in phase two clinical trials for improving the selectivity and reducing the side effects of cancer treatments.[1] This study investigates a novel light-controlled drug delivery strategy using photochemical internalization (PCI) to enhance the targeting and eradication of programmed death ligand-1 positive cells (PD-L1+), a key factor in tumor immune evasion.[2] Our research utilizes an immunotoxin, anti-PD-L1 conjugated with saporin, a ribosome-inactivating protein, combined with the PCI technology employing the photosensitizer fimaporfin (Amphinex®, $TPCS_{2a}$). The study shows that this approach significantly increases cytotoxicity in PD-L1+ tumor cells, specifically in lung cancer cell lines (H1975 and A549). Mechanistic insights reveal that PCI facilitates the endosomal and lysosomal escape of the immunotoxin into the cytosol, improving its efficacy.

*The study was supported by GAUK grant no. 1312220.*

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**BPT 9**

# EVALUATION OF MI-676 - A POTENT HUMAN CONSTITUTIVE ANDROSTANE RECEPTOR AGONIST FOR MAFLD

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Metabolism Associated Fatty Liver Disease (MAFLD) is a chronic condition resulting from several metabolic disturbances progressing through various stages from lipotoxic steatosis to eventual liver failure[1]. This can be mitigated by activation of the xenobiotic sensor, Constitutive Androstane Receptor (CAR), based on previous studies with murine agonist TCPOBOP, but there is no specific human CAR agonist accessible till date[2,3]. There are several genes that function as co-regulators of CAR in hindering cell death[4]; however, the molecular mechanisms are still unclear. The ongoing project aims to uncover function of novel human CAR agonist, fluorinated imidazo[1,2-a] pyridine derivative, MI676, in view of early apoptotic assay, along with molecular mechanism initiating through activation of primary regulatory transcription factors of CAR after stimulation, utilizing a differentiated HepaRG cell line, an appropriate alternative to human hepatocytes subjected to palmitic acid. Our findings reveal that MI676 has a protective impact by significantly inhibiting nuclear membrane breakdown and caspase activity. Moreover, we were able to identify the regulation of two key anti-apoptotic factors, MCL-1, and BCL-2, along with co-activators of CAR, GADD45B, and AKT, which ubiquitinate and degrade P53, thus possess a vital role in promoting cell survival. Drawing from the aforementioned results, we can connect the interrelated interactions between the dual signaling pathways, specifically the GADD45B-regulated Src/PP2A/MDM2 pathway and the NR1l3/AKT pathway.

*The study was supported by NETPHARM and GAČR.*

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# DISCOVERY OF A PXR ANTAGONIST MI891 AND THEIR ROLES IN HEPATIC GENE REGULATION

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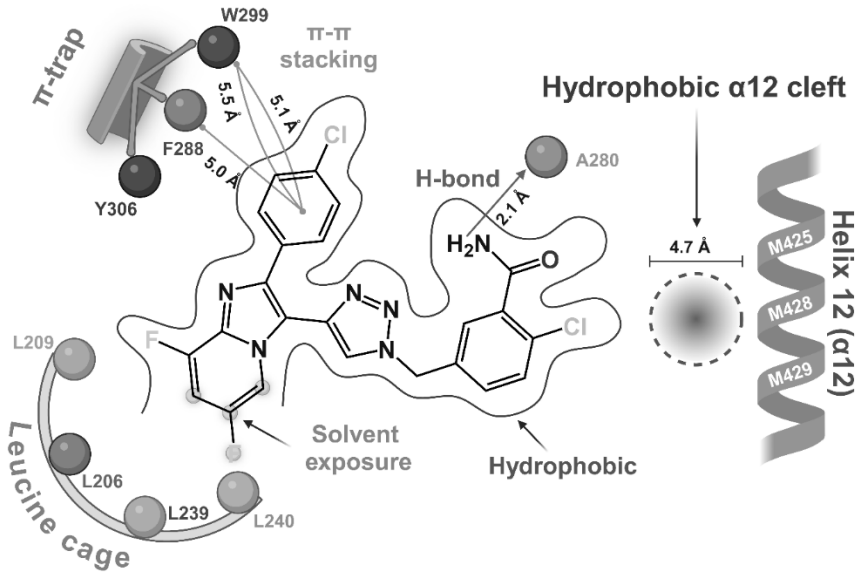
The pregnane X receptor (PXR) is an important regulator of drug metabolism and endogenous metabolic pathways. However, mechanistic insights into the effects of pharmacological inhibition using PXR antagonists on critical genes involved in both xenobiotic and endobiotic metabolism remain limited.

Here, we discovered a novel PXR antagonist MI891, which interacts with the ligand binding domain of PXR with nanomolar potency and is selective for PXR over its closely related receptors. Utilizing these tools, we investigated the regulation of key PXR target genes in differentiated hepatic HepaRG cells and human hepatocytes.

Our findings indicate that the PXR antagonism suppresses basal and rifampicin-induced expression of selected ADME genes. In addition, the PXR antagonists downregulate the expression of some key genes involved in gluconeogenesis, cholesterol homeostasis, bile acid synthesis, and proliferation in hepatocyte cells. Thus, the MI891 PXR antagonist represent novel tools for elucidating PXR's role in metabolic regulation, highlighting their potential as therapeutic candidates for metabolic diseases.

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**BPT 11**

# 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 inhibitors were introduced and became standard pharmacotherapy for Flt3-positive patients. Nevertheless, several mechanisms have been described to mediate resistance to these drugs leading to therapy failure and thereby driving an intensive effort in searching for new potent and specific Flt3 inhibitors. We aimed to evaluate 60 newly synthesized investigative compounds as potential Flt3 inhibitors. MTT assay revealed the three substances LG-2166, LG-2189, and LG-2192 with the highest antiproliferative activity on AML FLT3 mutated cell lines MOLM-13 and MV4-11 which significantly exceeded that in FLT3 non-mutated cells THP1 - specificity ratio (SR) to FLT3 mutation (MV4-11/THP1) of 155.7; 64.0; and 108.0 respectively). The proapoptotic effect of these compounds was further performed using Annexin V assay in MV4-11 and THP-1 cell lines. All three compounds showed superior proapoptotic effects induced in the mutated MV4-11 cells above that found in THP1 cells.

Among the three leading candidate drugs LG-2192 was identified as the most promising substance with superior antiproliferative activity and specificity to FLT3 mutated cells, which can be used as a lead compound for the synthesis of novel FLT3 inhibitors. These findings will be further verified using peripheral blood mononuclear cells from de novo AML patients.

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

# MATHEMATICAL MODELS TO HIGHLIGHT THE IMPACT OF NEGATIVE FEEDBACK LOOPS AND CIRCADIAN RHYTHMS ON PXR OSCILLATIONS

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The PXR (Pregnane X Receptor) nuclear receptor is a key regulator of metabolic enzymes and transporters, exhibiting oscillatory behavior under different regulatory mechanisms. This work develops mathematical models to explore two possible drivers of PXR oscillations: circadian rhythms and negative feedback loops. The first model demonstrates how rhythmic inputs from the circadian clock can affect PXR activity. The second model focuses on intrinsic negative feedback loops, where activated PXR downregulates its gene expression, generating oscillations sensitive to feedback strength and delay. A comparison reveals that circadian-driven oscillations are robust and entrained to external cycles, while feedback-induced oscillations are more sensitive to system parameters. These insights enhance our understanding of PXR dynamics and their implications for metabolism and drug response.

# DEVELOPMENT AND RESEARCH OF NOVEL COMPOUNDS TO COMBAT ANTIFUNGAL RESISTANCE

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Systemic and invasive fungal infections are a global health threat, with mortality rates ranging from 40% to 90%. Their treatment is challenged by a limited repertoire of available antifungals, many of which are associated with significant adverse effects or reduced efficacy due to the development of antifungal resistance.[1] To reflect this unpleasant situation, our study aimed to design new antifungal compounds and evaluate their effectiveness against clinically relevant fungal pathogens. Extended studies were performed on two candidate antifungal molecules, KMK-22 and SM-S-25, both polyhalogenated imines. These studies included antifungal activity testing against multidrug-resistant fungal isolates, time-kill kinetics analysis, determination of static vs. cidal action, and *in vivo* toxicity studies. The antifungal activity of selected compounds against both reference and clinical isolates of fungal strains ranged from 12.5 to 100 μM. In both compounds, the beneficial fungicidal effect was revealed, as well. These promising preliminary results provide a valuable rationale for further studies of other candidate polyhalogenated imines to cover the current limits in antifungal therapies.

*The study was supported by the Ministry of Health (CZE; NW24-05-00539) and SVV project No. 260 664.*

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**BPT 14**

# ANTI-ENDOGLIN MONOCLONAL ANTIBODIES AS A THERAPEUTIC APPROACH TO PREVENT THE PROGRESSION OF MASH: INSIGHT FROM *IN VIVO* AND *IN VITRO* MODELS

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Liver sinusoidal endothelial inflammation/dysfunction and fibrosis are key contributors to MASH development. TRC105 and M1043, anti-endoglin (ENG) monoclonal antibodies, specifically target ENG. This study hypothesized that anti-ENG treatment would prevent the progression of LSECs inflammation and fibrosis *in vivo* and *in vitro*. MASH was induced in male C57BL/6 mice fed a CDAA-HFD diet for 4 or 8 weeks. In the rescue study, mice were divided into three groups: control, MASH (CDAA-HFD+IgG), and rescue (CDAA-HFD+M1043), receiving rat IgG1 or M1043 (10 mg/kg). *In vitro*, human LSECs were activated with ox-LDL (50 μg/mL) and treated with TRC105 (300 μg/mL). MASH-induced LSECs inflammation was characterized by increased ENG, VCAM-1, and ICAM-1 expression and reduced VE-cadherin and p-eNOS/eNOS levels. M1043 treatment reversed these changes, prevented the upregulation of ENG, VCM-1 and ICAM-1, reduced liver fibrosis, and decreased the liver-to-body weight ratio.TRC105 also reduced ENG and VCAM-1 expression and monocyte adhesion in ox-LDL-activated LSECs. In conclusion, anti-ENG antibodies prevented LSECs inflammation and fibrosis progression in a MASH model and inhibited inflammation *in vitro*. Targeting ENG may represent a promising therapy for LSECs inflammation and liver fibrosis.

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**BPT 15**

# EFFECT OF GLIPIZIDE ON THE EXPRESSION AND FUNCTION OF ENDOGLIN AND RELATED BIOMARKERS OF ENDOTHELIAL DYSFUNCTION IN TYPE 2 DIABETES MELLITUS

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Endoglin (Eng) is a glycoprotein expressed in vascular endothelium and is associated with developing endothelial dysfunction and inflammation. Given its potential therapeutic effects on vascular function, this study aimed to investigate how glipizide affects the expression and function of endoglin and biomarkers of endothelial dysfunction in human coronary artery endothelial cells from patients with type 2 diabetes mellitus (D-HCAECs). Additionally, soluble endoglin (sEng) levels and monocyte adhesion to endothelial cells were analyzed. D-HCAECs were pretreated with 200 uM glipizide at various time points. Protein levels and THP-1 monocyte adhesion were assessed by flow cytometry. Levels of sEng were measured by ELISA, and PCR determined mRNA expression of genes. Premedication with 200 uM glipizide resulted in a decrease in endoglin expression. An increase in the expression of ICAM-1 was observed after 24 hours. Additionally, mRNA expression of the transcription factors Sp1, HIF-1a, and CCL2, were reduced after 16 hours of premedication with 200 uM glipizide. In contrast, increased mRNA expression of ICAM-1 and E-selectin was observed following 8 hours of premedication. Under the tested conditions, glipizide had no significant effect on monocyte adhesion or sEng formation. Glipizide was found to influence the expression of endoglin, and related adhesion molecules and markers associated with endothelial dysfunction. The reduction in endoglin expression suggests the potential therapeutic role of glipizide in regulating vascular function. Further detailed *in vitro* and *in vivo* studies are required to fully understand and confirm the effects of glipizide on endoglin and other aspects of endothelial dysfunction.

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**BPT 16**

# HUMANIZED MICE OVEREXPRESSING ENDOGLIN – AN ANIMAL MODEL SUITABLE TO INVESTIGATE THE EFFECT OF ENDOGLIN ON METABOLIC DYSFUNCTION-ASSOCIATED STEATOHEPATITIS

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Metabolic dysfunction-associated steatohepatitis (MASH) is a major risk factor for cardiovascular disease. Endoglin (ENG) is a transmembrane glycoprotein which is implicated in liver fibrosis. However, the role of ENG overexpression in MASH is unknown. This study aimed to establish an animal model to study ENG's impact in MASH. Ten-week-old transgenic mice expressing human L-endoglin (L-ENG+) on a CBAxC57BL/6J background and their wild-type (WT) littermates were fed either a chow or Choline-Deficient Amino Acid-defined high-fat diet (CDAA-HFD), which is a standard MASH-inducing diet, for eight weeks. Plasma biochemistry, liver enzymes, histology, and western blot analyses were performed to assess ENG expression and liver changes. Human ENG was expressed in the livers of L-ENG+ mice but absent in WT mice. Mouse ENG levels were comparable between L-ENG+ and WT mice, confirming human ENG as the primary difference. Both human and mouse ENG localized to liver sinusoidal endothelial cells, validating the model. The CDAA-HFD diet caused liver-to-body weight ratio increases (hepatomegaly) and elevated liver enzymes (alkaline phosphatase and alanine aminotransferase) in both groups, indicating liver damage.

These findings suggest that the L-ENG+ mouse model is a promising tool for studying ENG's role in liver disorders, including MASH.

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**BPT 17**

# CANNABINOIDS SUPPRESS LPS-MEDIATED INFLAMMATION IN HUMAN PLACENTAL EXPLANTS

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The increasing self-prescribed use of Cannabis sativa among pregnant women—driven by global legalization and a prevailing belief in the safety of natural products—has raised significant concerns regarding maternal-fetal health. Cannabis is often used to manage pregnancy-related symptoms such as nausea, vomiting, and anxiety; however, its precise impact on placental biology remains poorly understood. Notably, cannabinoids such as Δ9-tetrahydrocannabinol (THC), cannabigerol (CBG), cannabinol (CBN), and cannabivarin (CBV) have demonstrated the ability to modulate inflammation. This is particularly relevant in pregnancy, where both insufficient and excessive inflammatory responses can adversely affect maternal and fetal well-being. In this study, we investigated the anti-inflammatory effects of THC, CBG, CBN, and CBV at concentrations of 2.5, 10, and 20 μg/mL on human placental explants. Placental tissues were treated with exocannabinoids for 48 hours, followed by stimulation with 1 μg/mL lipopolysaccharide (LPS) for 4 hours to induce an inflammatory response. Key cytokines and inflammatory mediators were assessed through RT-qPCR and ELISA. We observed a significant downregulation of pro-inflammatory gene expression, including IL6, TNF-α, IL1B, IL18, NFKB, NLRP3, CASP1, and TLR4. These findings were further corroborated at the protein level, with reduced secretion of IL-6, IL-1β, and TNF-α, particularly at the highest cannabinoid concentrations. Our results suggest that cannabinoids can modulate inflammatory pathways in placental tissues, highlighting their potential impact on pregnancy outcomes.

*The study was supported by GAUK 170/50/225010 GACR 23-07094S*

**BPT 18**

# ENTECAVIR AFFECTS ON NUCLEOSIDE/NUCLEOTIDE METABOLISM IN THE PLACENTA: IMPLICATIONS FOR PREGNANCY

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Hepatitis B virus (HBV) can transmit from mother to fetus, and antiviral therapy is recommended for high viral loads. Entecavir (ETV), a first-line nucleoside analog for chronic HBV, is generally avoided in pregnancy due to insufficient safety data. The placenta is critical for fetal development, and our findings suggest nucleos(t)ides and adenosine signaling support stress adaptation. We hypothesize that ETV treatment may alter placental function and stress response mechanisms, with potential implications for fetal development. To test this hypothesis, we examined ETV (20 nM and 10 μM) in BeWo cells, placental explants (24-hour and 2-day treatments), and pregnant rats 0.1 mg $kg^{-1}$ daily from GD (gestation day) 1, with placentas collected on GD12, GD15, and GD20. RT-qPCR analysis in BeWo cells showed changes in gene ex unde under the treatment conditionsr the treatment conditionspressions related to adenosine metabolism, purine/pyrimidine pathways, and nucleoside transporters. Placental explants exhibited only minor changes, while rat placentas showed more profound gene expression alterations in all pathways compared to BeWo cells, varying across gestational stages. Western blot analysis confirmed dysregulation of adenosine receptors in rat placentas. In folow up experiments, using biomarkers of proliferation (*MKI-67*) and differention (*syncytin*), we found out that ETV can cause dysbalances in the placental development. Placental weight gain was significantly increased ($p < 0.05$) from GD15 to GD20 in the ETV-treated group, with fetal losses (1–3 per animal) observed in 4 out of 5 treated animals, compared to none in the controls.

These findings suggest that ETV modulates adenosine signaling and nucleos(t)ide metabolism, likely resulting in altered adaptability of the placenta to potential stress conditions.

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# INTERACTION OF NATURAL HYDROXYBENZOIC ACIDS WITH PHYSIOLOGICAL METALS IRON AND COPPER

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Benzoic acids are plant secondary metabolites which are commonly found in fruits and vegetables, especially in most berries including ripe fruits of *Vaccinium* species. Their daily intake is around 200 mg, depending on dietary habits[1,2]. They are also produced by human microbiota from other dietary phenolics[3]. The aim was to investigate the interactions of 8 natural benzoic acids with the biological metals iron and copper under different (patho)physiologically relevant pH conditions in terms of chelation, reduction, impact on the metal-based Fenton chemistry, and copper-based hemolysis. Only 3,4-dihydroxybenzoic acid behaved as a protective substance under all conditions. It chelated iron, reduced both iron and copper, and protected against the iron and copper-based Fenton reaction. Contrarily, 2,4,6-trihydroxybenzoic acid did not chelate iron and copper, reduced both metals, potentiated the Fenton reaction, and worsened copper-based hemolysis of rat red blood cells. The other tested compounds showed variable effects on the Fenton reaction. None of the tested compounds exhibited the ability to protect rat erythrocytes from copper toxicity. Interestingly, prooxidative benzoic acids mildly protected human erythrocytes against Cu-induced lysis. In conclusion, 3,4-dihydroxybenzoic acid seems to have a protective effect against copper and iron-based toxicity under different (patho)physiologically relevant conditions.

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# MASLD-INDUCED ALTERATIONS IN DETOXIFICATION SYSTEM

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The liver plays a major role in detoxifying endogenous and exogenous substances. Chronic liver diseases such as metabolic dysfunction-associated steatotic liver disease (MASLD) can modulate detoxification pathways.[1] This study aims to determine the activity of selected detoxification enzymes during MASLD progression. The C57BL/6N male mice were divided into two groups, one was fed with a standard diet (STD) and the other with a high fat, fructose, and cholesterol (FFC) diet from the age of 8 weeks for 1-6 months. The liver samples from both groups were collected at seven 4-week intervals. The subcellular fractions were prepared from liver samples and the specific enzymatic activities of several enzymes inc. superoxide dismutase (SOD), NAD(P)H:quinone oxidoreductase 1 (NQO1), and carbonyl reductase 1 (CBR1) were assessed spectrophotometrically using the appropriate substrates and obtained data were normalized to protein content. Feeding the FFC diet caused an increase in the activity of all observed enzymes, mainly in the latter time intervals, compared to age-matched STD-fed mice. The elevation in CBR1, NQO1, and SOD activities was most pronounced indicating a greater need for protection against oxidative stress-induced damage. This study shows not only the effect of the FFC diet on the activity of detoxification enzymes but also the changes in their activity during MASLD progression.

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**BPT 21**

# *VINCA MINOR* L. ALKALOIDS AND THEIR NEUROPROTECTIVE ACTIVITIES RELATED TO ALZHEIMER'S DISEASE AS MULTI-TARGET LIGANDS: *IN VITRO* AND *IN SILICO* STUDIES.

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The research is the ongoing study of the isolation of indole alkaloids from *V. minor*, and evaluation of their neuroprotective activity related to acetylcholinesterase (AChE), butyrylcholinesterase (BChE), monoamine oxidase A (MAO-A), tyrosinase (TYR), peroxisome proliferator-activated receptor gamma (PPAR-γ), prolyl oligopeptidase (POP), and glycogen synthase kinase-3β (GSK-3β) for anti-Alzheimer's multi-targeted effect. Interestingly, the alkaloidal extract of *V. minor* showed significant BChE inhibition of 61.68 ± 4.82% at 2 mg/ml. Henceforth, 90 g of the alkaloidal extract of *V. minor* was utilized to be fractionated further. So far, three indole alkaloids (+)-strictamine, (–)-minovincine, venoterpine and one non-alkaloidal compound 3,4-dehydrotheaspirone have been isolated by Flash chromatography and preparative TLC. Their structures were elucidated by MS, NMR, ECD and optical rotation. *In vitro* studies of (+)-strictamine and (–)-minovincine for the inhibition of AChE and BChE were conducted and their $IC_{50}$ values were found to be >100 μM for both the compounds. *In silico* studies of (+)-strictamine, (–)-minovincine were also performed to predict the ligand-protein interaction. Preliminary docking studies focused on TYR, PPAR-γ, and MAO-A showed interactions of these alkaloids with active sites of relevant targets. Hydrogen bonding, salt bridging, hydrophobic and van der Waals interactions were observed as modes of agonist and antagonist activity. Both compounds (+)-strictamine, (–)-minovincine were found to be noncytotoxic as well when tested on the human tumor and non-tumor cell lines.

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# INTERACTION OF HOMOCYSTEINE WITH HUMAN TRACE ELEMENTS

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Homocysteine (Hcy), a sulfur-containing amino acid, is a recognized risk factor for cardiovascular diseases.[1] Its interactions with trace elements, such as copper (Cu), iron (Fe), cobalt (Co), calcium (Ca), magnesium (Mg), and zinc (Zn), might be responsible for this phenomenon due to generation of oxidative stress. This study aimed to investigate the chelation and redox properties of Hcy with these essential metals and its influence on hydroxyl radical generation via the Fenton reaction driven by different metals. The study also evaluated the impact of Hcy on red blood cells (RBCs) in presence or absence of metals.

Chelation properties were assessed using various methods tailored to each metal, including the ferrozine method for Fe, the hematoxylin method for Cu, and the NNDSA (1-nitroso-2-naphthol-3,6- disulfonic acid disodium salt) method for Co. The Fenton reaction was analyzed using high-performance liquid chromatography (HPLC) coupled with coulometric detection to monitor hydroxyl radical production. Additionally, hemolysis was assessed by lactate dehydrogenase activity monitoring.

Preliminary results demonstrated that Hcy is a potent chelator for several metals, particularly Co and Cu, with significant modulation of their redox activity. While Hcy reduced hydroxyl radical generation in some metal systems, it exacerbated oxidative stress in others, highlighting a dual antioxidant-prooxidant role. Notably, Hcy-metal complexes exhibited varying effects on RBC lysis, depending on the metal and pH conditions.

This study underscores the complex interplay between Hcy and trace elements, revealing its potential to modulate oxidative stress and cellular damage. These findings contribute to understanding Hcy's role in pathological conditions, particularly those involving metal-catalyzed oxidative stress.

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# EFFECT OF DIRECT ANTICOAGULANTS ON PLATELET-MONOCYTE AGGREGATES OCCURRENCE IN PATIENTS WITH METABOLIC DISEASES

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Enhanced platelet reactivity is observed in patients with cardiovascular and metabolic conditions.[1] Hyperlipidemia, diabetes mellitus (DM) and metabolic syndrome are diseases with a high incidence, particularly in the so-called developed countries.[2] These patients present a markedly increased risk of suffering from cardiovascular events. Platelet-monocyte aggregates (PMA) have emerged as reliable biomarkers for the early detection of various cardiovascular diseases. These aggregates occur only in the presence of activated platelets. In this study, we analyzed the occurrence of PMA by flow cytometry in a total of 115 patients with 3 metabolic diseases (familial hypercholesterolemia - FH, diabetes mellitus type 1 - DM T1 and metabolic syndrome – MS/DM T2). Detected PMA for each patient group were compared to those of 50 healthy subjects. In all patient groups, PMA occurrence was significantly higher than in healthy individuals and, when compared to the detected activated platelets, good correlations were obtained. In order to evaluate the influence of anticoagulant treatment *ex vivo*, samples were individually treated with 4 direct anticoagulants (rivaroxaban, apixaban, dabigatran and argatroban), all at an equimolar concentration of 1 μM. Interestingly, the treatment with anticoagulants decreased PMA incidence when compared to untreated samples, with rivaroxaban and dabigatran showing the highest effectivities. Our results prove that PMA are already detected in patients with metabolic cardiovascular risk factors.

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# THE WINS AND FAILURES ON CURRENT DOCKING METHODS TESTED ON THE FLEXIBLE ACTIVE SITE OF CYP PROTEINS

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CYP-mediated mechanism plays large part in metabolizing of drugs with estimates, that 75% of all commercially available drugs are metabolized by only 6 out of 57 CYP enzymes in humans[1]. The proteins responsible for this process, heme-containing cytochromes P450, are a challenge for docking with rigid protein approximation which shows limited reliability[2,3].

This study[4] evaluates open-source docking engines in a high-throughput manner using a dataset of 128 ligands. Four distinct engines (GALAXYDOCK2 HEME, VINA, GNINA, and RosettaFoldAll-Atoms)—are tested in both redocking and crossdocking simulations. In redocking, we dock ligands into proteins with the appropriate protein conformation for the ligand. In crossdocking, the protein is folded but lacks conformational information, necessitating conformational changes. Thus, crossdocking is more relevant for practical applications. We also introduce system-specific metrics focused on the iron atom of heme to compare results. During the talk, we will present our simulation workflow, discuss metrics, conclusions and close the presentation with current challenges in docking to P450 cytochromes.

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