72nd CZECH-SLOVAK
PHARMACOLOGICAL DAYS

ABSTRACT BOOK

Faculty of Medicine UPJŠ in Košice
Technology and Innovation Park

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72nd CZECH-SLOVAK PHARMACOLOGICAL DAYS

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PREFACE

It is our great pleasure to welcome you to the **72nd Czech-Slovak Pharmacological Days**, held this year in the vibrant and culturally rich city of **Košice**, at the **Faculty of Medicine of Pavol Jozef Šafárik University**.

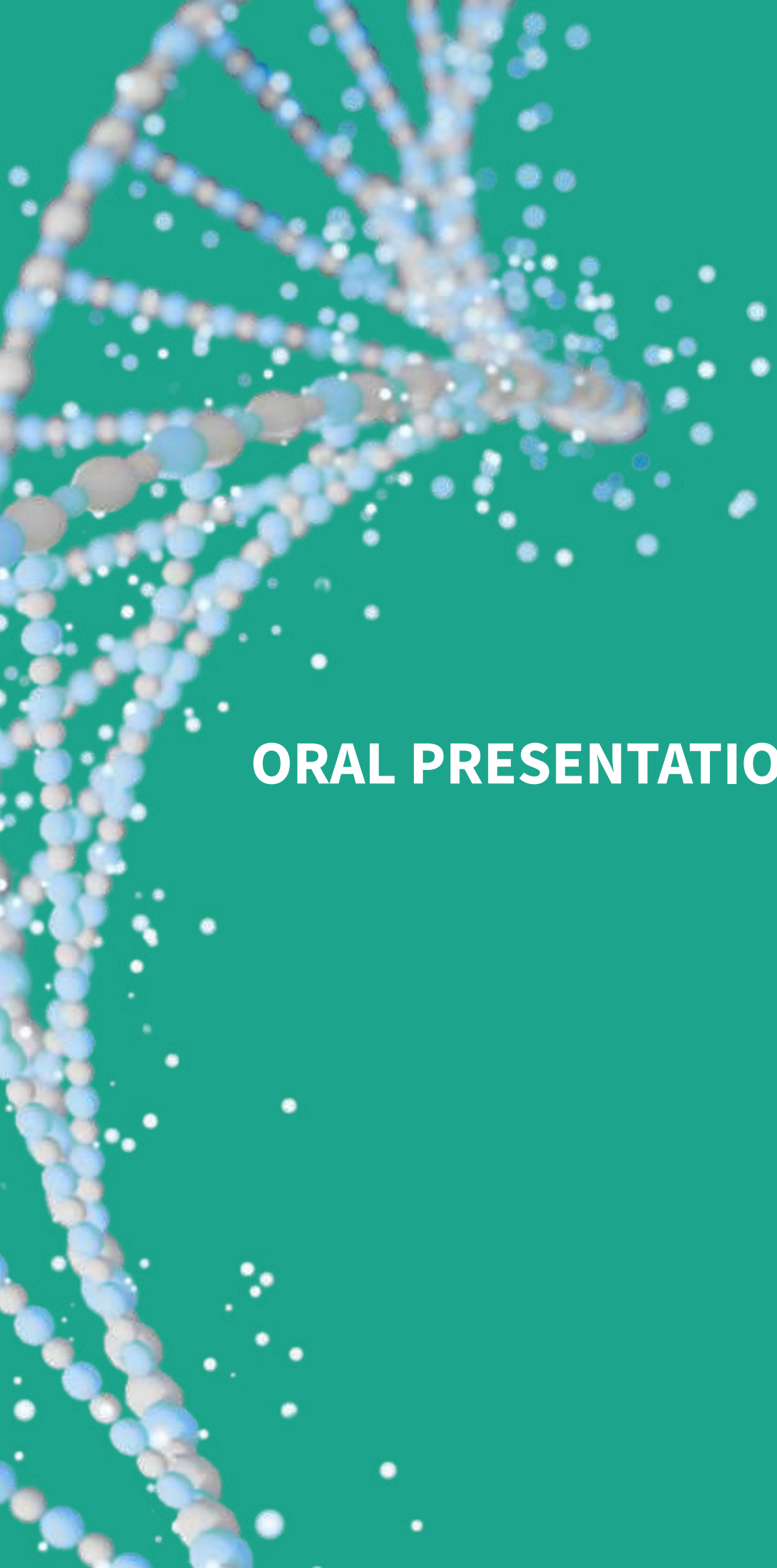
This long-standing scientific conference continues its tradition of fostering collaboration, sharing knowledge, and supporting progress in the fields of **pharmacology, pharmacy, toxicology, and biomedical sciences**. The 2025 meeting brings together **distinguished researchers, clinicians, educators, and students** from the Czech Republic, Slovakia, and beyond, united by a common goal—to advance our understanding of pharmacological science and improve human health.

The scientific program reflects the diversity and depth of current pharmacological research. It includes sessions focused on **experimental pharmacology, inflammation, oncology, respiratory pharmacology, neuropharmacology,** and **personalized medicine**, as well as **innovative approaches in drug development and therapy**. This year, special attention is given to **young scientists**, whose enthusiasm and novel perspectives enrich our community and represent its future.

We hope that this meeting will provide not only valuable insights and fruitful discussions but also new collaborations and friendships. In addition to the academic program, we invite you to explore the **historical landmarks, remarkable culture, and warm hospitality of our city**.

We extend our sincere thanks to all participants, speakers, organizing and scientific committees, and institutional partners whose efforts and dedication made this event possible.

Welcome to Košice, and to the **72nd Czech-Slovak Pharmacological Days**.



ORAL PRESENTATIONS

ENHANCEMENT OF METHOTREXATE THERAPY BY LOTUS (*NELUMBO NUCIFERA*) IN EXPERIMENTAL ARTHRITIS: SUPPRESSION OF INFLAMMATORY AND OXIDATIVE STRESS MARKERS

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Background: *Nelumbo nucifera*, also known as the "sacred lotus", has long been used in Asian medicine for its therapeutic properties. The aim of our study was to examine the bioactive potential of *N. nucifera* leaf extracts and powders at various doses, with special emphasis on their anti-arthritic and anti-inflammatory properties, which could be relevant to the treatment of rheumatoid arthritis as well as other chronic inflammatory diseases.

Material/Methods: Rats with adjuvant-induced arthritis were used in this study to assess the effects of *N. nucifera* leaf extract and powder. Three doses of leaf extract (100, 250, and 500 mg/kg) and 500 mg/kg dose of leaf powder were administered *per os* as monotherapies in pilot study. The selected 500 mg/kg of *N. nucifera* leaf extract was further administered in pivotal study in combination with methotrexate (MTX, 0.3 mg/kg, p.o., twice a week), because of observed positive effects of this extract on biometric parameters and on the modulation of plasmatic IL-17A and MMP-9 levels.

Results: The most important results from the pilot experiment with different doses and forms of *N.nucifera* leaves were: a) anorectic effect demonstrated in change of body weight and b) anti-arthritic effect most pronounced for the dose of 500 mg/kg of *N. nucifera* extract in comparison with its other doses and in comparison with leaves powder evaluated at the same dose demonstrated in change of hind paw volume and of arthritic score, as well as in plasmatic levels of IL 17A and MMP-9. In pivotal experiments, we measured the same parameters as in the pilot experiment and it was confirmed that co-administration of *N. nucifera* to methotrexate improves its antiarthritic and anti-inflammatory therapeutic effect. Moreover, the antioxidative effect was also observed in liver tissue (mRNA expression of heme oxygenase 1 and catalase), erythrocytes (catalase activity) for the combination therapy, which is an additional benefit.

Conclusions: The combination treatment of methotrexate and *Nelumbo nucifera* leaf extract showed improved efficacy, and the notable anti-arthritic benefits could be the basis for further preclinical research.

Keywords: *Nelumbo nucifera* leaves extracts, rheumatoid arthritis, experimental arthritis, methotrexate, combination therapy, biometric parameters

Acknowledgment: VEGA 2/0079/24, VEGA 2/0091/23, VEGA 2/0126/23; SAS-VAST bilateral project (responsible: Dr. Bauerova and Dr. KhanhNgocj Pham)

EFFECT OF SELECTED SUBSTANCES ON ANGIOGENESIS INVESTIGATED BY THE *IN OVO* METHOD

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Background: Angiogenesis is a biological process in which blood vessels are formed under physiological and pathological conditions. Physiological angiogenesis begins during fetal development and persists postnatally and plays an important role in growth. Pathological angiogenesis, caused by tumors, heart attack, wound healing, and chronic inflammation, is highly aberrant, with irregular distribution, irregular branching, and nonphysiological connections between arteries and veins. Given the importance of vessel growth in tumor progression and metastasis, inhibition of angiogenesis has been investigated *in ovo* as a therapeutic prospect for cancer treatment. *In ovo* studies using avian embryos represent a valuable alternative to traditional *in vivo* experiments. The alternative animal model presented is cost-effective, readily available, reproducible, reliable, and also meets the requirements of the 3Rs - Reduce, Refine, and Replace.

Material/Methods: We investigated the effect on angiogenesis of the natural substance β-escin, *Phellodendron amurense* Rupr. tincture and synthesized indole phytoalexin on the chorioallantoic membrane of the chicken embryo *in ovo*.

Results: The synthetic indole phytoalexin MB-653 had neither a stimulatory nor an inhibitory effect on angiogenesis, which was demonstrated by measuring up to 4 parameters - the size of the vascular zone, the number of branching vessels, the total length of the vasculature, and the average thickness of the vessels. Inhibition of angiogenesis was determined for β-escin and tincture of *Phellodendron amurense* Rupr. by suppressing proliferation and migration.

Conclusions: Our results suggest that *Phellodendron amurense* Rupr. extract and β-escin, by their anti-angiogenic and anti-proliferative effects, could be used in the therapy of different types of cancer.

Keywords: angiogenesis, *in ovo*, β-escin, *Phellodendron*, phytoalexin

Acknowledgment: This work was supported by grant VEGA 1/0373/2 and VEGA 1/0436/24.

TARGETING MITOCHONDRIA: INDOLE CHALCONE MODULATES OXIDATIVE STRESS AND ENERGY METABOLISM IN BREAST CANCER.

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Background: Chalcones are bioactive compounds with significant therapeutic potential due to their well-documented anti-inflammatory, antioxidant, antimicrobial, and anticancer properties. In this study, we investigated the effects of a novel synthetic chalcone derivative, 11d, on breast cancer (BC) cell lines.

Material/Methods: The effect of chalcone 11d on the energy metabolism of MCF-7 (luminal A), MDA-MB-231 (triple-negative) cancer cells and non-tumorigenic MCF-10A cells was evaluated at concentrations of 16 µmol/L (IC20) and 24 µmol/L (IC30). To obtain results, various methods were employed, including CN-PAGE and Western blotting. Mitochondrial respiration was assessed using the high-resolution Oxygraph-2k system. Mitochondrial morphology was visualized by confocal microscopy. Oxidative and carbonyl stress markers were quantified in cell culture supernatants to evaluate the redox status.

Results: Chalcone 11d selectively impaired mitochondrial function in breast cancer cell lines, particularly MCF-7. It caused an early and progressive decrease in mitochondrial membrane potential and disrupted mitochondrial morphology, leading to fragmentation and dysfunction, while healthy MCF-10A cells remained largely unaffected. Complex I activity increased in cancer cells, suggesting stress response. An oxygen consumption was significantly reduced, indicating impaired oxidative phosphorylation in cancer cells. Western blot analysis showed altered expression of mitochondrial dynamics proteins, particularly in MCF-7 cells. Oxidative stress was increased in tumour cells, as reflected by increased AOPP and lipid peroxidation. While antioxidant defense was increased in healthy breast tissue cells, it remained relatively intact in tumour cells, or showed a slight decrease in MDA-MB-231 cells. Chalcone 11d also induced carbonyl stress, with reduced AGE levels in cancer cells and changes in glutathione status reflecting redox imbalance. In contrast, healthy cells maintained redox homeostasis.

Conclusions: These findings suggest that chalcone 11d exerts selective cytotoxicity in breast cancer cells by disrupting mitochondrial function and redox balance, supporting its potential as an anticancer and antimetastatic agent.

Keywords: cancer cells, mitochondria, oxidatives stres, energy metabolism, chalcones

Acknowledgment: This work was supported by the Scientific Grant Agency of the Ministry of Education of the Slovak Republic under Contract No. VEGA 1/0498/23.

CHALCONES – PROMISING MOLECULES IN THE FIGHT AGAINST RESISTANT TUMOR CELLS

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Background: The enhanced expression and activity of the ABCB1 (P-glycoprotein) membrane transporter, which facilitates drug efflux, are regarded as an important factor in tumor cell multidrug resistance. Recent studies have identified chalcones and their derivatives as promising agents capable of modulating the activity of this transporter. Our work focused on the several newly synthesized acridine-substituted chalcones – namely (2E)-3-(Acridin-4-yl)-1-(4-methoxyphenyl)prop-2-en-1-one (4B), (E)-3-(acridin-9-yl)-1-(2,6-dimethoxyphenyl) prop-2-en-1-one (1C) and (2E)-3-(Acridin-4-yl)-1-(3,4,5-trimethoxyphenyl) prop-2-en-1-one (4E), examining their *in vitro* activity on cell viability in tumor cells overexpressing the ABCB1 transporter. In addition, their selectivity, ability to inhibit efflux, and potential for drug interaction were evaluated.

Material/Methods: The viability of both cancerous (COLO 320 colorectal adenocarcinoma cells overexpressing ABCB1) and non-cancerous (BJ-5ta human foreskin fibroblasts) cell lines was evaluated using the methylthiazoltetrazolium assay. Flow cytometry was employed to verify the expression and functionality of ABCB1 in the tumor cells. To determine whether the compounds selectively targeted cancer cells, the selectivity index (SI) was computed. The ability of chalcone derivatives to influence ABCB1-mediated drug efflux was examined using a fluorescence-based method. Furthermore, the interaction between the most potent anti-efflux chalcone and the ABCB1 substrate doxorubicin was assessed through combination index (CI) analysis.

Results: Our analyses demonstrated that the chalcones effectively suppressed the viability of tumor cells overexpressing active form of ABCB1, with IC_{50} values below 10 µM. In contrast, significantly higher IC_{50} values were observed in non-tumor cell line, indicating their selective antiproliferative effect on tumor cells (SI > 3). Among the tested compounds, 1C exhibited the strongest anti-efflux activity and showed a synergistic interaction with doxorubicin (CI < 1).

Conclusions: Chalcone-acridine hybrids are promising molecules, showing considerable potential, as they not only reduce the viability of resistant cancer cells, but also act selectively against tumor cells and inhibit ABCB1-mediated drug efflux, thereby enhancing the potency of cytostatic treatment.

Keywords: ABCB1, chalcone, efflux, P-glycoprotein, resistance

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INNOVATIVE APPROACHES IN THE DIAGNOSIS AND TREATMENT OF MYCOBACTERIAL INFECTIONS

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Background: Mycobacterial infections, including tuberculosis (TB) and non-tuberculous mycobacteria (NTM), pose significant diagnostic challenges, especially in pediatric patients. Emerging methods, such as droplet digital PCR (ddPCR) for stool-based TB diagnosis and whole-genome sequencing (WGS) for detecting resistance in NTM, offer promising advancements for improved diagnosis and tailored treatment.

Material/Methods: A novel dd-PCR-based method was developed and optimized using biological samples (blood, stool, gastric aspirate) obtained from patients (0-18 years old) hospitalized at the National Institute of Pediatric Tuberculosis and Respiratory Diseases. WGS was performed on 142 isolates of NTM, including 19 rare NTM, 29 isolates of Mycobacterium abscessus, and 94 isolates of Mycobacterium avium, to identify mutations that encode resistance.

Results: Novel dd-PCR method on stool showed 100% sensitivity compared to conventional PCR on gastric aspirate. Moreover, TB was detected in two clinically diagnosed patients from whole blood and gastric aspirate solely using the ddPCR method. Sequencing data analysis confirmed resistance to macrolides in 22 (15.9%) isolates, predominantly due to mutations in the erm gene. One isolate harboured the mutation in the rrs gene associated with resistance to aminoglycosides.

Conclusions: Our findings demonstrate that the newly developed ddPCR method can effectively replace conventional RT-PCR techniques, thereby eliminating the need for invasive GA sampling in pediatric TB diagnosis. WGS analysis indicated that up to 15% of NTM isolates exhibit resistance to macrolide antibiotics, which are crucial for treating these infections. However, the actual prevalence of resistant strains may be higher, as genetic resistance in NTM remains insufficiently investigated.

Keywords: tuberculosis, resistance, diagnostics, non-tuberculous mycobacteria, management

Acknowledgment: This research was funded by Grant APVV-18-0084, Grant APVV-22-0342, Grant VEGA-1/0093/22, Grant VEGA- 1/0049/25

INHIBITION OF ABC TRANSPORTERS BY NATURAL SUBSTANCE DERIVATIVES: FOCUSING ON MOLECULAR MECHANISMS

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Background: Chalcones, precursors for flavonoids in plants, have been shown to possess diverse biological effects, such as antitumor effects and the ability to inhibit efflux mechanisms. There is a scarcity of research regarding their effect on multidrug resistance driven by ATP-binding cassette (ABC) efflux transporters. In this study, we performed *in vitro* experiments to investigate the modulation of selected ABC transporters by an acridine-based chalcone (E)-3-(acridin-9-yl)-1-(2,6-dimethoxyphenyl) prop-2-en-1-one derivative (1C).

Material/Methods: We employed a cell line model consisting of colorectal adenocarcinoma cell line COLO 320 overexpressing ABCB1 (P-glycoprotein) and ABCC1 (multidrug resistance-associated protein 1). The ability to inhibit the protein expression of ABC transporters and their regulatory proteins – galectins 3 and 8, was assessed using the Western blot technique. Furthermore, differential scanning calorimetry was used to study the effect of chalcone 1C on the thermal properties of a model lipid bilayer prepared from the saturated phospholipid dipalmitoylphosphatidylcholine.

Results: Our study demonstrates that acridine-based compounds, represented by the 1C chalcone derivative, can modulate the ABC transporters. Exposure to the 1C significantly lowered ABCB1 protein levels and partially reduced ABCC1 protein expression in COLO 320, while the expression of galectin regulatory molecules remained largely unchanged. Furthermore, differential scanning calorimetry revealed an effect of 1C on membrane integrity, which could be associated with decreased efflux activity of the membrane ABC transporters.

Conclusions: In conclusion, the 1C chalcone derivative, an acridine-based compound, effectively modulates the ABCB1 and ABCC1 transporters by reducing their protein expression and significantly altering the physicochemical properties of lipid bilayers, indicating its ability to interact with cellular membranes. These findings suggest that 1C chalcone has the potential to overcome ABC-mediated drug resistance without affecting the expression of selected galectin regulatory molecules.

Keywords: ABC transporter, calorimetry, colorectal adenocarcinoma, chalcone, galectin

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THE IMPORTANCE OF RHO KINASE IN ALLERGIC ASTHMA: FINDINGS FROM A GUINEA PIG EXPERIMENTAL MODEL

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Background: Allergic asthma is a chronic inflammatory disorder of the airways characterized by reversible bronchoconstriction, airway hyperresponsiveness (AHR), mucus overproduction, and persistent inflammation. Despite the availability of inhaled corticosteroids and bronchodilators, a significant subset of patients remains symptomatic or develops steroid resistance. Therefore, novel therapeutic strategies targeting alternative pathways involved in asthma pathogenesis are urgently needed. Among these, Rho-associated coiled-coil forming protein kinases (ROCKs) have emerged as promising targets due to their involvement in smooth muscle contraction, inflammatory cell recruitment, and airway remodeling.

In this study, we investigated the therapeutic potential of selective and non-selective ROCK inhibitors in an ovalbumin-induced guinea pig model of allergic asthma. We compared the effects of two non-selective inhibitors, GSK429286A and hydroxyfasudil, and a ROCK inhibitor preferentially targeting ROCK2, H1152, with both healthy controls and ovalbumin-sensitized untreated animals (positive control) on key pathophysiological features of asthma.

Material/Methods: Our experimental model involved sensitization and repeated ovalbumin challenges to induce asthma-like features, including AHR, airway inflammation, and remodeling. The inhibitors were administered intraperitoneally or orally in defined dosing regimens. Their effects were evaluated thorough several parameters, including *lung function tests* assessed by measuring of specific airways resistance after histamine inhalation; *cough response* evaluated after citric acid inhalation; *tissue homogenates analysis* included quantification of interleukins (IL) and cytokines using Bio-plex 200 system, while levels of transforming growth factor β (TGF- β), collagen (COL) 3 and 5, and other markers were assessed by ELISA; *histological examination* of lung tissue was performed to assess COL deposition, goblet cells quantification around bronchioles while *ciliary activity* was evaluated by measuring ciliary beat frequency (CBF).

Results: All three inhibitors significantly reduced AHR and cough response compared to positive controls. Cytokine analysis demonstrated a marked reduction in Th2 cytokines (IL-4, Il-5 or IL-13) and fibrotic markers (TGF-β and COL), with the most pronounced effects observed in the non-selective inhibitors. Histological assessment revealed decreased COL deposition and mucus hypersecretion, indicating attenuation of airway remodeling. CBF remained unaffected across all groups.

Conclusions: Our findings underscore the pivotal role of ROCK signaling in allergic asthma pathogenesis. Non-selective ROCK inhibitors, in particular, exhibited strong anti-inflammatory and anti-remodeling effects in this guinea pig model, supporting their potential as novel therapeutics for Th2-driven asthma subtypes resistant to standard treatments.

Keywords: allergic asthma, guinea pig, ROCK inhibitors, inflammation, remodeling

Acknowledgment: This work was supported by the grants: APVV-19-033, APVV-23-0261, VEGA 1/0042/24, and VEGA 1/0060/25

PHARMACOKINETICS OF RITUXIMAB IN VARIOUS GLOMERULAR DISEASES

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Background: Pharmacokinetics of monoclonal antibodies is not influenced by a decrease in glomerular filtration rate or impaired liver functions. Nevertheless, it can be expected that it is altered in the case of nephrotic syndrome with non-selective proteinuria, which frequently develops in some glomerular diseases. Presented study deals with the pharmacokinetics of rituximab (RTX) in various glomerulopathies with a different range of proteinuria and aims to describe the covariates that influence RTX pharmacokinetics.

Methods: Between May 2020 and June 2023, all patients treated with RTX for glomerular diseases were repeatedly examined during outpatient visits on the Nephrology department of the General University Hospital in Prague for RTX blood levels and levels of anti-RTX antibodies. Based on measured RTX levels population pharmacokinetic model was developed by means of Monolix Suite software. The dosing that will ensure the same RTX exposure for all patients was proposed based on this model.

Results: The final model was based on 445 levels measured in 185 patients. We found that RTX clearance significantly increases with an increase in proteinuria and in the presence of anti-RTX antibodies. Another important independent factor for RTX CL was a type of glomerulopathy, with CL significantly greater in membranous nephropathy (MN), lupus nephritis (LN) and focal-segmental glomerulosclerosis (FSGS) when compared with ANCA-associated vasculitis (AAV) and minimal change disease (MCD). CL was further influenced by length of the treatment and body weight of the patients. Volume of distribution was influenced only by body weight.

Conclusion: Proteinuria has been proven to be an important covariate influencing RTX clearance and in accordance with the literature, we also proved that RTX clearance is increased in patients with detectable anti-RTX antibodies. The study further proved that patients with glomerular diseases with less reliable clinical response to RTX treatment (MN, LN and FSGS) have faster RTX clearance and that the diagnosis itself is an independent factor influencing RTX clearance even when proteinuria is already taken into account. We proposed a novel RTX dosing schedule for patients that may be undertreated with RTX due to increased CL.

Keywords: membranous nephropathy, lupus nephritis, focal-segmental glomerulosclerosis, ANCA-associated vasculitis, minimal change disease

Acknowledgement: This study was supported by the research initiatives of the Ministry of Health of the Czech Republic (RVO-VFN 64165) and the Ministry of Education – Czech Republic grant COOPERATIO 207034 Internal Disciplines and Pharmaceutical Sciences.

HEART FAILURE WITH PRESERVED EJECTION FRACTION IN RELATIONSHIP TO MICRORNA – A PILOT STUDY

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Background: Heart failure with preserved ejection fraction (HFpEF) is multifaceted syndrome with complex aetiology and it occupies the specific position within patients with heart failure with alarmingly rising tendency. The risk of „HFpEF phenotype“ development grows up with comorbidities such as metabolic syndrome, diabetes mellitus type 2, arterial hypertension, dyslipidaemia, and sleep apnoe syndrome. These diseases induce systemic, chronic inflammation of low intensity, microvascular dysfunction, metabolic stress, tissue ischemia and fibrosis. Identification and therapy of patients with HFpEF includes many difficulties. The recent studies revealed that microRNA (miRNA) play crucial role in HFpEF pathogenesis regulation. Detection of biomarkers including miRNA can contribute to early diagnostics and subsequent adequate therapy of these patients.

Methods: In randomizely prospectively kohort of 100 patients with HFpEF we will evaluate specific subtypes of microRNA (1, 133a, 133b, 499, 208a, 209b) in correlation with laboratory biomarker of heart failure (N-terminal pro brain natriuretic peptide) and echocardiographic features of left ventricle diastolic dysfunction (E/e´, septal e´, lateral e´, left atrial volume index and tricuspid valve velocity) for the purpose of exact identification of HFpEF patients, to predict prognosis and progression of HFpEF and to select optimal pharmacotherapy. Specific types of miRNA in patients will be compared with healthy probands to identify subtypes of miRNA which could be pathognomonic for HFpEF phenotype.

Results: Perspective of the study is to predict prognosis and progression of HFpEF phenotype on the base of specific microRNA subtypes detection in correlation with laboratory biomarker NTproBNP and echocardiographic parameters of left ventricle diastolic dysfunction. The second outcome is to evaluate optimal pharmacotherapy consists of sodium glucose co transporter 2 inhibitors (SGLT2I) and mineralocorticoid receptors antagonists (MRA) in relationship to specific microRNA - the change of microRNA after six months therapy.

Conclusions: Identification of specific subtypes of microRNA could be helpful in early diagnostics of patients with HFpEF as well as in delaying heart failure symptoms progression by adjusting optimal pharmacotherapy, which includes the therapy of comorbidities and also therapy targeted on pathophysiology of HFpEF (regression of inflammation and fibrosis), mainly SGLT2I and mineralocorticoid receptor antagonists.

Keywords: heart failure, microRNA, ejection fraction, microvascular damage, therapy

ASSESSMENT OF 24,25-DIHYDROXYVITAMIN D3 PRODUCTION ACROSS INCREASING VITAMIN D SUPPLEMENTATION DOSES IN HEALTHY YOUNG ADULTS

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Background: The increasing interest in vitamin D supplementation has raised concerns regarding its optimal dosing, particularly due to the potential risk of vitamin D toxicity. However, emerging evidence suggests that vitamin D toxicity may be overestimated, as additional metabolic detoxification pathways have been identified. One such pathway involves 25-hydroxyvitamin D3 24-hydroxylase, which facilitates the inactivation of vitamin D. In this study, we investigated the levels of 24,25-dihydroxyvitamin D3 ($24,25OH_2D_3$) in relation to vitamin D supplementation.

Material/Methods: A total of 38 healthy young adults (mean age: 22 years) underwent increasing vitamin D supplementation over a two-year period during winter months using an oil-based formulation, with doses ranging from 1,000 IU to 8,000 IU. Liquid chromatography-mass spectrometry was employed to quantify serum concentrations of 25-hydroxyvitamin D3 ($25OHD_3$) and $24,25OH_2D_3$. Body composition, including body mass index (BMI), body fat percentage, fat mass, and skeletal muscle mass, was assessed using the InBody S10 system.

Results: A strong correlation was observed between $25OHD_3$ and $24,25OH_2D_3$ levels. The production of $24,25OH_2D_3$ was most pronounced at moderate supplementation doses but remained stable even at the highest dose of 8,000 IU. The $25OHD_3/24,25OH_2D_3$ ratio was higher in male participants and significantly correlated with BMI.

Conclusions: The metabolic inactivation of vitamin D, as reflected by $24,25OH_2D_3$ production, persists even at supplementation doses of 8,000 IU, with the ratio of active vitamin D3 to its metabolite remaining relatively stable throughout the supplementation period. These findings suggest that vitamin D supplementation with doses as high as 8,000 IU per day does not overwhelm the body's metabolic capacity and may be considered safe in healthy young adults for addressing seasonal vitamin D decrease.

Keywords: Vitamin D, vitamin D toxicity, 25-hydroxyvitamin D, 24,25-dihydroxyvitamin D

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MUCOCILIARY CLEARANCE IN ALLERGIC ASTHMA

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Background: In Th2-high asthma, airway inflammation is associated with epithelial features of airway remodelling, including goblet cell metaplasia, bronchial gland hypertrophy, altered mucin expression, and impaired mucociliary clearance (MCC), a key airway defence mechanism reliant on coordinated ciliary activity and proper mucus rheology. Standard asthma therapy has a limited impact on remodelling processes, contributing to increased disease severity and frequent exacerbations. This review summarises potential therapeutic targets aimed at restoring MCC and reinforcing epithelial barrier function.

Material/Methods: Ciliary Beat Frequency (CBF) was assessed in tracheal epithelial cells collected by brush biopsy from healthy, allergen-sensitised, and pharmacologically treated guinea pigs. Treatments included standard therapy, agonists or antagonists targeting calcium signalling or other asthma-related pathways, mucolytics, and selected natural compounds. Cells were maintained in RPMI at 37 °C, placed on pre-warmed slides, and imaged using a high-speed digital camera attached to an inverted phase-contrast microscope (40×). Ciliary motion was analysed using Ciliary Analysis software. MUC5AC expression was evaluated in lung homogenates using either ELISA or immunohistochemistry, depending on the experimental design.

Results: A significant increase in CBF was observed after treatment with CRAC channel blockers, T2R receptor agonists, and the combination of LABA with glucocorticoids. T2R agonists also reduced MUC5AC levels. In contrast, TMEM16A agonists significantly reduced CBF while improving mucus viscosity. Rho kinase modulation had no significant effect on either CBF or MUC5AC expression. Although polyphenols showed anti-asthmatic potential under allergic conditions, their effect on CBF was mostly neutral or inhibitory. However, the mucolytic agent erdosteine significantly enhanced ciliary activity along with additional favourable effects.

Conclusions: Our research confirms the key role of calcium and cAMP in regulating ciliary movement, with calcium also contributing to the suppression of pathological mucus production and reduced viscosity in the airways. These findings highlight both mediators as promising pharmacological targets for enhancing MCC in allergic asthma. Agents with multiple mechanisms of action remain of particular therapeutic interest.

Keywords: mucociliary clearance, ciliary beat frequency, calcium, cAMP, MUC5AC

Acknowledgment: This research was supported by the grants APVV-19-0033, APVV-23-0261, VEGA 1/0060/25, and VEGA 1/0042/24.

NON-INVASIVE THERAPEUTIC DRUG MONITORING OF ANTI-SEIZURE MEDICATION

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Background: Therapeutic drug monitoring (TDM) is essential for optimizing anti-seizure medication (ASM) therapy in epilepsy, ensuring seizure control while minimizing adverse effects and drug interactions. While traditional venous blood collection for TDM may be stressful, emerging micro-sampling methods, particularly Dried Blood Spot (DBS), Saliva or dried saliva spot (DSS), offer less invasive alternatives. This study aimed to develop and validate an analytical method for the determination of ASM (levetiracetam, lamotrigine, valproate, lacosamide, eslicarbazepine, carbamazepine, and cenobamate) in alternative matrices, such as saliva, dried saliva spot and dried blood spots.

Methods: The samples were subjected to extraction, evaporation, and reconstitution in 15% acetonitrile containing 0.1% formic acid. A Kinetex C18 Polar column was used for liquid chromatographic separation, and MS in ESI+ mode was used for detection and quantitation using an isotopically labelled internal standards. Saliva and DBS samples from patients treated with lamotrigine analysed by the developed method were compared to plasma concentrations measured by the hospital's accredited laboratory.

Results: The method underwent basic validation accordingly with the EMA guidelines. Thirteen parallel samples were analysed by LC-MS, and the Pearson correlation analysis revealed statistically significant correlations between DBS and plasma measurements, as well as between saliva and plasma measurements.

Conclusions: Our results indicate a promising potential for these alternative matrices in clinical TDM applications.

Keywords: anti-seizure medication; therapeutic drug monitoring, alternative matrices, dried blood spot, saliva

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MARINE-DERIVED SPHINGOLIPIDS AS PROMISING AGENTS AGAINST CERVICAL CARCINOMA

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Background: Cancer remains a significant and growing threat to public health. Despite the widespread use of chemotherapeutics, conventional therapies often face limitations, including suboptimal efficacy, adverse side effects, and safety concerns. As a result, there is an increasing demand for novel and innovative anticancer agents. In recent decades, natural products have gained considerable attention as potential candidates for anticancer drug discovery. Unlike traditional drug development from terrestrial sources, the marine environment has only recently been recognized as a rich and promising reservoir of structurally unique and biologically active secondary metabolites capable of modulating various signaling pathways involved in tumor growth and progression. Sphingolipids represent a family of lipid that play crucial roles in maintaining membrane integrity, and regulating key cellular processes such as proliferation, differentiation, cell cycle progression, and cell death. In this study, we focused on the sphingolipid analog 2-epi-jaspine B, a stereoisomer of jaspine B originally isolated from the marine sponge *Jaspis sp.*

Material/Methods: HeLa cells (cervical adenocarcinoma) were used as the experimental model. The antiproliferative effects were evaluated using MTS metabolic assays and CellTrace™ proliferation assays. Flow cytometry and Western blot analysis were employed to investigate the mechanism of action, focusing on proteins involved in apoptosis and sphingolipid metabolism. Gene expression of sphingolipid-related enzymes was evaluated using RT-PCR.

Results: Compound M1 inhibited tumor cell proliferation with an IC_{50} value of 6.5 µM in HeLa cells. The sphingoid base M1 induced apoptosis characterized by externalization of phosphatidylserine, loss of mitochondrial potential, cytochrome c release, caspase activation and subsequent cleavage of PARP. These mitochondrial events were associated with the modulation of Bcl-2 family members. Additionally, an increase in ceramide synthesis was observed.

Conclusions: Our findings demonstrate that the antiproliferative effect of M1 in HeLa cells involves the activation of the intrinsic (mitochondrial) apoptotic pathway. Our data unveiled, for the first time, that modulation of sphingolipid metabolism and ceramide accumulation contributes to M1-mediated cell death in a cervical cancer model.

Keywords: sphingolipids, apoptosis, antiproliferative effect, marine sponges

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SLAMF5 AND SLAMF7 RECEPTORS AS DIAGNOSTIC MARKERS AND POTENTIAL THERAPEUTIC TARGETS IN HAIRY CELL LEUKEMIA

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Background: Hairy cell leukemia (HCL) is a rare type of B-cell chronic lymphoproliferative disorder characterized by prominent splenomegaly and progressive pancytopenia. It accounts for less than 2% of all leukemia cases. Diagnosis relies on cytological examination and flow cytometry to identify characteristic B-cell antigens, such as CD19, CD20, CD22, and HCL-specific markers including CD11c, CD25, CD103, and CD123. Identification of novel antigens expressed on malignant HCL cells may provide important diagnostic, prognostic, and therapeutic opportunities.

Material/Methods: This study analyzed the expression of all nine SLAMF receptors on B-lymphocytes from bone marrow samples of 4 patients with HCL using multiparametric flow cytometry. The expression levels of SLAMF receptors were compared between healthy and pathological B-cells.

Results: The analysis revealed altered expression of two out of nine studied SLAMF receptors in pathological HCL B-cells compared to healthy B-cells. Specifically, pathological cells showed increased expression of SLAMF5 and SLAMF7. No significant differences were observed in the expression of the remaining SLAMF receptors.

Conclusions: Our findings demonstrate that SLAMF5 and SLAMF7 are significantly overexpressed on pathological HCL B-cells compared to healthy B-cells. Their overexpression could aid in the identification of malignant cells and serve as diagnostic markers or therapeutic targets. Further studies are required to validate these preliminary findings and to investigate the potential role of SLAMF receptors in HCL therapy.

Keywords: SLAMF receptors, hairy cell leukemia, flow cytometry, B-lymphocytes, diagnostic markers

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OPTIMISATION OF TUBERCULOSIS TREATMENT USING PHARMACOGENETICS AND THERAPEUTIC DRUG MONITORING

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Background: Tuberculosis (TB) is considered one of the deadliest infectious diseases in the world. Its prevalence is also increasing in Slovakia, including the paediatric population. Increasing resistance to first-line antituberculosis drugs poses a serious therapeutic problem. Community transmission, poor adherence, and inappropriate treatment regimens may contribute to the resistance development. In addition to the presence of resistant strains of *Mycobacterium tuberculosis*, individual patient characteristics may also influence the treatment success. These include age, comorbidities, drug interactions as well as genetic predisposition.

Material/Methods: Blood samples were collected from the patients diagnosed with tuberculosis and hospitalized at the National Institute of Tuberculosis, Lung Diseases and Thoracic Surgery in Vyšné Hágy. Their plasma was used for the measurement of the levels of isoniazid, ethambutol, and pyrazinamide. Subsequently, DNA was isolated and the most frequent polymorphisms of the major metabolizing enzyme N-acetyltransferase 2 (NAT2) were analyzed.

Results: According to the genotype, the phenotype (metabolizer status) of each patient was determined and compared to the plasmatic concentrations of isoniazid at two and six hours after administration. Adjustments were made for isoniazid dose, patients' age, and body mass index (BMI) to account for interindividual variability.

Conclusions: These preliminary findings highlight the potential role of pharmacogenetics and therapeutic drug monitoring in optimizing TB treatment.

Keywords: tuberculosis, pharmacogenetics, therapeutic drug monitoring

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O6-BENZYLGUANINE DECREASES FIBROTIC MARKERS VIA TGF-β NON-CANONICAL PATHWAYS, LEADING TO DECREASED EPITHELIAL-MESENCHYMAL TRANSITION IN A MODEL OF UNILATERAL URETERAL OBSTRUCTION

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Background: O6-benzylguanine (BG) is an MGMT (O6-methylguanine-DNA-methyltransferase) inhibitor and is used only in combination with methylating agents to treat chemoresistant tumors. In our previous studies, we observed decreased proliferation of vascular smooth muscle cells along with lower expression of α-SMA (α-smooth muscle actin). Therefore, we hypothesized that BG may also inhibit fibroblast proliferation, thus reducing fibrosis development.

Material/Methods: Experiment was conducted on 12-week-old Wistar rats. The rats were randomized into three groups: SHAM – the control group, with a standard surgical procedure performed; UUO – the group with Unilateral Ureteral Obstruction performed on the left kidney; and BG – the group with Unilateral Ureteral Obstruction, treated with O6-benzylguanine at a dose of 30 mg/kg every 24 hours for 7 days. After 7 days, animals were euthanized and the left kidneys were harvested. Proteins were isolated using RIPA buffer, separated by SDS-PAGE (12%) and analysed by western blot.

Results: In the BG-treated group, we observed a significant decrease in the levels of: pJNK/JNK, β-catenin, vimentin, α-SMA, E-cadherin and HMGB1. The results also showed decreased levels of signaling molecules such as pERK/ERK, pAKT, N-cadherin, pSMAD2, NF-kB. No changes were observed in the levels of pMEK/MEK, pp38, MnSOD.

Conclusions: Based on our results, BG possesses antifibrotic properties, as we observed decreased expression of α-SMA. Moreover, it can interfere with TGF-β non-canonical pathways included in fibrogenesis. One of these pathways is the JNK pathway, which is important for the upregulation of proinflammatory and growth factors. JNK is also capable of directly phosphorylating SMAD2/3 proteins, thereby supporting TGF-β canonical pathway. The significantly decreased β-catenin is part of the Wnt (Wingless-type MMTV integration site family) pathway, which is important for the upregulation of epithelial-mesenchymal transition proteins and the inhibition of the antifibrotic pSMAD7 protein, resulting in increased activity of pSMAD2/3 proteins. These facts may explain why the level of pSMAD2 is also decreased. PI3K/AKT belongs to another TGF-β non-canonical pathway that leads to the overexpression of extracellular matrix proteins and can indirectly activate β-catenin through the inhibition of GSK-3β. The attenuation of these pathways by BG leads to decreased levels of markers of epithelial-mesenchymal transition, such as vimentin, N-cadherin or main prefibrotic marker, α-SMA, which are all important in the fibrotic process.

Keywords: fibrosis, TGF-β, UUO, O6-benzylguanine, EMT

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RISKS OF IONIC IMBALANCE WHEN SUPPLEMENTING WITH INCREASING DOSES OF VITAMIN D

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Background: Vitamin D toxicity is rare and can be observed with very high doses of vitamin D. The most serious complication is the formation of calcium phosphate crystals in the kidneys, which can lead to kidney failure. We supplemented the group of patients with increased doses of cholecalciferol and monitored ion levels to ensure the safety of the therapy.

Material/Methods: Thirty-five volunteers participated in a two-season pilot study conducted from October to April to avoid sunlight-induced vitamin D3 synthesis. The participants used oil-based drops of cholecalciferol, increasing their dose from 1000 to 2000, 4000, and then 8000 IU daily for 60 days with a 30-day break. Vitamin D (25OHD, calcidiol) calcium, phosphorus, magnesium and parathormone levels were determined and evaluate.

Results: We observed a positive correlation of calcium with 25OHD levels and a negative correlation of parathormone with 25OHD levels. These trends remained within the reference ranges of the individual parameters. None of the participants experienced any clinical symptoms of vitamin D overdose.

Conclusions: All dosage regimens of cholecalciferol administered in our study were safe for patients. Monitored trends in ions and parathormone levels remained within reference ranges.

Keywords: Vitamin D, supplementation safety, calcium, phosphate, parathormone

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ANTISEIZURE DRUG RISK IN PREGNANCY

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Background: Epilepsy is one of the most common chronic neurological disorders characterized by repeated seizures, which require long-term or long-life treatment. The aim of pharmacological treatment is the elimination of seizures alongside minimizing adverse drug effects and drug interactions and thus improvement in quality of life. A special approach in drug choice decisions is required in women throughout the life stages - from childhood through menarche, childbearing age, pregnancy, childbed, breastfeeding and after menopause as well.

Key Findings / Discussion: The careful family planning, including consulting obstetrician and neurologist is crucial in women with epilepsy from the very moment of childwish. Patient´s compliance and adherence to therapeutic recommendations are of key importance. Antiseizure medications pose certain teratogenic risk nevertheless, the drug risk is smaller compared to the teratogenic risk of uncontrolled generalized seizures itself. Particular anticonvulsants are associated with different degrees of teratogenic risk, hence, monotherapy should be preferred in most cases. Anyway, the risk of major congenital malformations in women on monotherapy is approximately 2-4 times higher compared to the background population, resp. healthy comparators. The highest risk of causing congenital defects is associated with valproates (valproic acid, sodium valproate), especially doses exceeding 1000 mg/die, phenobarbitone or primidone. Follow-up studies focused on the drug dose relationship of congenital malformations did not reveal increased risk in subgroups treated with lamotrigine and levetiracetam. Regarding other „newer“ anticonvulsants (brivaracetam, lakosamid, perampanel, vigabatrin) there is not enough data obtained to draw

Conclusions: Hormonal changes occurring throughout a woman's life can influence and be influenced by seizure mechanisms and antiepileptic drugs. More effective antiepileptic drugs, therapeutic plasma monitoring (plasma concentrations) of the drug, and better understanding of the risk factors for pregnancy have made it possible for women with epilepsy to have healthy, normal children. The lowest effective dose of the most appropriate antiepileptic drugs should be used (lamotrigine and levetiracetam are considered recommended), aiming for monotherapy where possible. Most pregnancies are uneventful in women with epilepsy, and most babies are delivered healthy with no increased risk of obstetric complications in women.

Keywords: drug risk, pregnancy, epilepsy, woman

CLINICAL-PHARMACEUTICAL INTERVENTIONS FOR IDENTIFICATION OF EARLY STAGE OF COGNITIVE DISORDERS

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Background: The global ageing of the population is contributing to the dramatical increase of the prevalence of cognitive impairment (CI) and dementia, which are becoming one of the biggest challenges for health systems and for society as a whole. For this reason, various strategies are being sought in order to slow down this trend and prevent their occurrence. Known modifiable risk factors that contribute to the development of CI include cardiovascular diseases, obesity, metabolic syndrome, type 2 diabetes, lifestyle, social isolation, depression, but also the use of at-risk medication, which can be effectively managed by the pharmacist.

Material/Methods: Cognitive screening was realised using The Montreal Cognitive Assessment screening tool (expressed by MoCA score). The presence of modifiable risk factors for CI was evaluated by calculation of cardiovascular risk factors and ageing (CAIDE score); assessment of at-risk medication use by the Anticholinergic burden scale (ACB).

Results: 323 patients (mean age 72.9±9.0 years) with polypharmacy (6.5± 5.0 medicines/person, at-risk medication rate -ACB score 1.5± 2.0). underwent a cognitive screening realised within pharmaceutical care. Older elderly patients (age 75+; N=118; 37%) achieved lower mean MoCA score 18.4±6.0 points than younger elderlies (age 60-74; N= 205; 63 %) 23.6±4.3 points ($P< 0.0001$). Both groups, patients with at-risk medication (ACB score 3+) and 75+ patients achieved lower MoCA score ($P< 0.0001$) within cognitive screening realised by pharmacists in community pharmacy.

Conclusions: Early identification of at-risk patients with potential cognitive impairment using a simple cognitive test within advanced pharmaceutical care setting may improve early interventions and physician-indicated targeted treatments.

Integrated pharmaceutical care involving regular medications analysis and consequent optimization of pharmacotherapy according to current criteria may also be an effective tool to minimize adverse effects on cognitive health.

Keywords: cognitive screening, medication use, mental health, clinical pharmacy

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DIHYDROISOXAZOLE-DERIVED COMPOUND DHI1 INDUCES AUTOPHAGIC CELL DEATH IN PROMYELOCYTIC LEUKEMIA CELLS

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Background: Leukemia is the 13th most common cancer and the 10th leading cause of cancer-related death worldwide. In 2022, an estimated 487,000 new cases and approximately 305,000 deaths were reported. It is also the most common childhood cancer, accounting for 30–40% of pediatric malignancies. This study evaluates the isoxazole derivative DHI1 and its mechanism of action against leukemia cells, with a focus on its role in autophagic cell death and its effects on key molecular targets.

Material & Methods: Jurkat (T-lymphoid) and HL-60 (promyelocytic) leukemia cell lines were used to evaluate cell viability, lysosomal activity, and protein expression profiles. Cells were treated with DHI1 and/or chloroquine, followed by MTT assays to assess viability. For lysosomal staining, LysoTracker™ Deep Red and Hoechst 33342 were used, and imaging was performed using OLYMPUS FV-1000 BX61 confocal microscope. Western blotting was conducted to analyse the expression of proteins involved in autophagy. Additionally, NF-κB phosphorylation was analysed using the AlphaScreen SureFire p-NF-κB Kit. Statistical analyses were performed using GraphPad Prism 9.

Results: In Jurkat cells, co-treatment with DHI1 and chloroquine reduced viability compared to DHI1 alone, suggesting that DHI1 does not induce autophagy-mediated cell death. In contrast, in HL-60 cells, the combination increased viability, indicating autophagy-dependent cell death. Lysosomal dye accumulation was observed specifically in HL-60 cells after DHI1 treatment. Additionally, DHI1 downregulated autophagy-related proteins (AKT, mTOR) and inhibited NF-κB phosphorylation.

Conclusions: Our findings indicate that DHI1 induces autophagy-dependent cell death in HL-60 cells and suppresses NF-κB activation. These results highlight DHI1 as a promising candidate for targeting myeloid leukemias through dual modulation of apoptotic and autophagic pathways.

Keywords: isoxazole, leukemia, autophagy, cell death

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EFFECT OF CANNABIDIOL ON THE CELL PROLIFERATION AND ITS POTENTIAL AS AN ANTITUMOR DRUG

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Background: Many preclinical models have provided evidence that phytocannabinoids are cytotoxic for tumor cells and cannabidiol (CBD) belongs among the most promising phytocannabinoids for cancer therapy. Our goal was to investigate the potential CBD has to target tumor cells whilst having a minimal or no effect on healthy cells.

Material/Methods: Two model cell lines were used for the experiment: human embryonic kidney cells (HEK293T ATCC® CRL-3216™) that served as non-pathological cells and a neuroblastoma cell line that served as a carcinoma model (SH-SY5Y ATCC® CRL-2266™). Four different concentrations of CBD (1mg/ml in methanol) were tested (6.25, 12.5, 25 and 50 µg/ml). The methods utilized included the xCELLigence system (real-time cell analysis) for the continuous monitoring of cell adhesion and proliferation, an XTT end-point assay to colorimetrically assess changes in cellular metabolic activity (MA) and a Scratch test to evaluate changes in cell migration.

Results: We observed that all concentrations had a significant negative effect on the proliferation of the HEK293T cells and the SH-SY5Y cells ($p < 0.05$), except for the lowest concentration 6.25 µg/ml on the SH-SY5Y cells ($p > 0.05$). The EC50 values for CBD were calculated using the xCELLigence system data after 72 h of treatment. The EC50 (half maximal effective concentration) recorded for HEK293T cells was 8.03 µg/ml and 6.57 µg/ml for SH-SY5Y cells, which shows that SH-SY5Y cells are more sensitive to the cytotoxic effect of CBD. Measurements of the MA showed that the lowest concentration (6.25 µg/ml) reduced the MA of the SH-SY5Y cells but increased the MA of the HEK293T cells ($p < 0.05$). The lowest concentration, therefore, displayed selective cytotoxicity towards the cancer cells, whereas all the other concentrations reduced the MA more of the healthy cells than the cancer cells. Scratch assay revealed that after 48 h of incubation, 6.25 µg/ml reduced cell migration of the HEK293T cells, whereas the SH-SY5Y cells were not affected.

Conclusions: These results show that CBD has the potential to be used for the future development of targeted alternative cancer therapy and supports the view of using lower treatment dosages. However, further research is still needed to determine the specific dose and treatment duration for each tumor type and animal species.

Keywords: cytotoxicity, anti-tumor, proliferation, metabolic activity, scratch assay

Acknowledgment: This work was supported by the National Laboratory of Pesticides of the University of Veterinary Medicine and Pharmacy in Košice, Slovakia.

UNCOVERING TAS2R-LINKED MECHANISMS OF AMAROGENTIN IN ASTHMA: FROM *IN SILICO* SCREENING TO *IN VIVO* ANIMAL MODEL

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Background: Recent studies have revealed that bitter taste receptors (TAS2Rs) play important roles beyond taste perception, particularly in regulating inflammatory processes within the airways. Amarogentin, a plant-derived secoiridoid, has been reported as an agonist of multiple TAS2Rs. This study integrates computational, cellular, and animal models to explore the therapeutic potential of amarogentin in allergic airway diseases.

Material/Methods: The BitterX platform was used to predict amarogentin's affinity for TAS2R subtypes. Its effects on degranulation and calcium signaling were assessed in LUVA mast cells. In guinea pigs sensitized with ovalbumin, oral amarogentin was evaluated for its effects on airway responsiveness, mucociliary clearance, cough reflex, smooth muscle contractility, Th2 cytokine levels, mucin production, and airway remodeling.

Results: Amarogentin demonstrated a potent inhibitory effect on mast cell degranulation through calcium-dependent pathways linked to bitter taste receptor activation. In the asthma model, it reduced bronchial hyperresponsiveness, enhanced mucociliary function, and attenuated key markers of Th2-mediated inflammation, while preserving cough reflex integrity. No significant impact on airway remodeling markers was observed.

Conclusions: Our findings support the anti-inflammatory potential of amarogentin in allergic airway diseases. By modulating TAS2R-mediated calcium signaling, amarogentin effectively reduced airway hyperresponsiveness and inflammation without influencing structural airway changes, suggesting its promise as an immunomodulatory therapeutic agent.

Keywords: amarogentin, mast cells, allergic asthma, inflammation**.**

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FIBOFLAPON: MORE THAN JUST A LEUKOTRIENE SYNTHESIS INHIBITOR? AN *EX VIVO* ANALYSIS OF FIBOFLAPON EFFECT ON MAST CELL-MEDIATED TRACHEAL RESPONSES

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Background: This study was conducted retrospectively, following an *in vivo* comparison of fiboflapon, a leukotriene synthesis inhibitor, and montelukast, a cysteinyl leukotriene receptor 1 ($CysLT_1$) antagonist, in house dust mite (HDM)-sensitized guinea pigs. Fiboflapon completely suppressed the early asthmatic response to allergen challenge, whereas montelukast failed to demonstrate a comparable effect. These findings suggested that fiboflapon may exert additional effects beyond leukotriene inhibition, potentially involving histamine modulation.

Material/Methods: Tracheal tissues from non-sensitized and HDM-sensitized female guinea pigs were used in this study. Following euthanasia, the airways were flushed with ice-cold Krebs-Henseleit buffer. The trachea was rapidly dissected and sectioned into circular segments. A potential antagonistic effect of fiboflapon was evaluated in non-sensitized tissues, while tissues from sensitized animals were used to assess its effect on histamine release after HDM exposure, both acutely and after 96-hour incubation with fiboflapon. All experiments were performed using a myograph.

Results: No antagonistic effect of fiboflapon was observed in tracheal tissues from non-sensitized guinea pigs. Fiboflapon also did not exert any acute effect on the tracheal response to cumulative doses of HDM in tissues from sensitized guinea pigs. However, 96-hour incubation with fiboflapon modified the tracheal response to KCl. Moreover, a rightward shift of the dose-response curves (DRCs) for both histamine and carbachol, with a marked reduction of the contraction upon HDM challenge, was observed. Assuming that the reduced contraction was due to reduced effect of histamine, post-hoc analysis taking account for the reduction of potency of histamine indicated that a part of the attenuated HDM-induced contraction was due to a decrease in histamine release.

Conclusions: While fiboflapon itself does not display acute antagonistic effects on responses to histamine, prolonged exposure attenuated the responsiveness to multiple contractile agonists, with a particularly strong effect observed for the HDM response. The present experiments, along with consecutive analyses, suggest that the efficacy of fiboflapon in suppressing early allergic responses in the *in vivo* study depends on both a decreased potency of histamine and diminished histamine release, which was possibly amplified when fiboflapon was administered to guinea pigs over several days.

Keywords: fiboflapon, histamine, house dust mite, trachea, myograph.

PEDIATRIC TUBERCULOSIS: TREATMENT RISKS, DRUG RESISTANCE, AND TRANSMISSION PATTERNS

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Background: Pediatric patients represent a vulnerable population in whom the diagnosis and treatment of tuberculosis (TB) involve several specific challenges and risks. As children often present with non-specific clinical symptoms and a pauci-bacillary form of the disease, the use of advanced molecular diagnostic methods, such as whole-genome sequencing (WGS), may be preferred. In this study, we focused on pediatric patients to study the genotypic resistance and transmission of TB, as well as the adverse effects associated with treatment.

Methods: Culture samples were obtained from the National Reference Laboratory for Mycobacteria in Slovakia and the Czech Republic. The isolates were analyzed using WGS to assess resistance patterns and transmission chains. Adverse effects were evaluated based on medical reports.

Results: Phylogenetic and cluster analyses confirmed the Euro-American lineage in most patients, while the East Asian lineage was also identified. Resistance to at least one drug was detected in six patients. Cluster analysis revealed nine clusters comprising 24 out of 37 patients. Adverse effects associated with first-line antituberculosis drugs occurred in 68 out of 123 pediatric patients, with the most frequently reported being hyperuricemia (90%) and hepatopathy (43%).

Conclusions: Combining genetic analysis with epidemiological data can significantly enhance the identification of transmission events and support more targeted public health interventions. Furthermore, monitoring adverse effects during the treatment of the pediatric population is essential, as children may exhibit different pharmacokinetic profiles than adults, which can be associated with changes in treatment efficacy.

Keywords: pediatric tuberculosis, WGS, drug resistance, transmission chains, adverse effects

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BIOLOGICAL EFECT OF *STEREOCAULON GRANDE* EXTRACT AND ITS SECONDARY METABOLITE, LOBARIC ACID, ON HCT116 COLORECTAL CARCINOMA CELLS

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Background: The idea of utilizing lichens originates from traditional medicine, where their medicinal properties have been recognized since the Middle Ages. Modern research confirms their broad spectrum of biological activities, including anticancer, anti-inflammatory, antioxidant, and analgesic effects, making them promising candidates for pharmaceutical applications. Lichen secondary metabolites have been shown to modulate key signaling pathways involved in carcinogenesis through antiproliferative, cytotoxic, antioxidant, and proapoptotic mechanisms.

Material/Methods: The anticancer effects of *Stereocaulon grande* (SG) extract and lobaric acid (LA) were evaluated on the HCT116 colorectal cancer cell line based on prior screening analyses. Cell viability was assessed using the resazurin assay, supplemented by proliferation assays (e.g., CellTrace Yellow). Apoptotic activity, changes in mitochondrial membrane potential, effects on the cell cycle, and DNA damage in response to oxidative stress were analyzed using flow cytometry, fluorescence microscopy, and western blotting. All analyses were performed in triplicate at 24, 48, and 72 hours after treatment.

Results: *Stereocaulon grande* (SG) extract and lobaric acid (LA) exhibited dose- and time-dependent anticancer activity in HCT116 colorectal cancer cells. SG and LA inhibited cell proliferation and colony formation, induced apoptosis through mitochondrial dysfunction (loss of membrane potential and cytochrome *c* release), and promoted oxidative stress (increased production of ROS, RNS, and O_2^{-}), leading to DNA damage. Co-treatment with the antioxidant NAC partially mitigated the pro-oxidative effects. Consistent with these results, cell cycle arrest was also observed at the G2/M phase.

Conclusions: The *Stereocaulon grande* extract and lobaric acid induce apoptosis in colorectal cancer cells *via* activation of the intrinsic mitochondrial pathway, accompanied by proliferation inhibition through G2/M cell cycle arrest. These findings highlight the pharmacological potential of *Stereocaulon* species in the prevention and treatment of colorectal cancer.

Keywords: lichen, *Stereocaulon*, lobaric acid, apoptosis, colorectal cancer

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1-METHOXYISOBRASSININ AS A MODULATOR OF THE TUMOR MICROENVIRONMENT WITH A FAVORABLE SAFETY PROFILE

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Background: Indole phytoalexins, secondary metabolites from *Brassicaceae* plants, exhibit anticancer properties such as inhibition of proliferation, induction of apoptosis, modulation of gene expression, and autophagy induction. Our previous studies demonstrated strong antiproliferative activity of 1-methoxyisobrassinin against gynecological cancer cell lines. Preclinical evaluation must assess not only efficacy but also safety and selectivity, commonly using cancer–fibroblast co-cultures and blood element testing. *In ovo* chicken embryo models provide complementary data on vascular effects, embryotoxicity, malformations, administration routes, and gene expression during development, helping to identify compounds appropriate for safe anticancer use.

Material and Methods: Selectivity of 1-methoxyisobrassinin (MB-591) was evaluated using co-cultures of FaDu hypopharyngeal carcinoma cells and SCCF cancer-associated fibroblasts (CAFs). Selective cytotoxicity was assessed by immunofluorescence (vimentin and cytokeratin staining) and MTT assay. Hemolytic activity and lymphocyte cytotoxicity were tested on bovine blood samples. *In ovo* studies assessed vascular irritation on the chorioallantoic membrane (CAM) and embryotoxicity following injections into somites, aorta, and yolk. Gene expression changes in autophagy markers (ATG7, Beclin 1) were evaluated using whole-mount *in situ* hybridization (WISH).

Results: MB-591 demonstrated selective cytotoxicity towards FaDu carcinoma cells without affecting SCCF fibroblasts. No hemolytic activity or cytotoxicity towards lymphocytes was observed. *In ovo* studies revealed no vascular irritation, embryotoxicity, or teratogenic effects following administration. Gene expression analysis confirmed no alterations in ATG7 or Beclin 1 levels, indicating no disruption of autophagic processes.

Conclusions: MB-591 exhibits selective anticancer activity along with a favorable safety profile, supporting its potential for further development in anticancer drug discovery.

Keywords: indole phytoalexins, tumor microenvironment, toxicological profile, hemotoxicity, embryotoxicity

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TUBERCULOSIS IN SLOVAKIA – EPIDEMIOLOGICAL ASPECTS AND ACTUAL SITUATION IN DIAGNOSTICS AND THERAPY

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Background: Despite constant progress in the diagnosis of Mycobacterium tuberculosis (MTB) infection, conventional culture methods are still primarily used. However, their variable sensitivity and duration complicate the correct onset of the treatment regimen.
Whole genome sequencing (WGS) can significantly contribute to early diagnosis and to increased treatment efficiency, especially in patients with resistant forms of tuberculosis (TB). The identification of new gene variants in key genes associated with resistance, including the new generation antituberculosis drugs delamanid and bedaquiline, has been described as another potential benefit of fast and comprehensive molecular-genetic diagnostics. The relevance of gene polymorphisms needs to be evaluated based on correlation with the results of the phenotypic drug susceptibility testing and the Xpert MTB/RIF genotypic method. In cooperation with the WHO, the newly identified mutations have been recently included in the latest versions of a mutation catalog. Screening all patients coming to Slovakia and the Czech Republic due to war in Ukraine is being performed, as the incidence of resistant TB in Ukraine is the highest in Europe. The target has been reached by introducing WGS in routine TB diagnostics, as the first laboratory in the Central European region. Another contribution has been the development of analytical methods for determining the levels of first and second-line antituberculosis drugs using LC/MS, enabling an individualization of TB therapy. Furthermore, the monitoring of changes in clinical and laboratory parameters depending on the level and type of resistance is being performed, enriching knowledge on the pathogenesis of MTB infections and changes in the immunological profile of treated patients, and thus allowing personalized pharmacotherapy with proper and effective antituberculotics.

Conclusions: The implementation of advanced methods like WGS and LC/MS in tuberculosis diagnostics and monitoring yields significant improvements in accuracy, speed, and treatment personalization, which is particularly crucial in the context of the spread of drug-resistant strains.

Keywords: tuberculosis, antituberculotic, diagnostics, resistance, epidemiology

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PHARMACOKINETICS OF INHALED AND INTRAVENOUS ZANAMIVIR IN A RAT MODEL OF ACUTE LUNG INJURY

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Background: Zanamivir is a neuraminidase inhibitor used to treat influenza A and B. Due to its low oral bioavailability, it is usually administered as an inhalable powder or via intravenous infusion. However, it remains unclear whether the zanamivir pharmacokinetics (PK) is altered under conditions of inflammatory lung damage, such as acute lung injury (ALI). ALI leads to changes in the permeability of the alveolar-capillary membrane, which may affect drug absorption and distribution. This study aimed to evaluate the PK and pulmonary penetration of zanamivir in healthy rats and rats with induced ALI.

Material/Methods: PK studies were performed in healthy rats and rats with induced ALI. ALI was induced by intratracheal administration of lipopolysaccharides (LPS) from E. coli (5 mg/kg). Zanamivir (5.1 mg/kg) was administered either intravenously (IV) or via nebulised inhalation (INH). To evaluate absolute bioavailability, a two-period study was performed in which the same animals received IV zanamivir in one period and INH zanamivir in a second period. Changes in systemic exposure to inhaled zanamivir were evaluated using a two-period study, where the rats were healthy in the first period, and ALI was induced prior to the second period. Permeation of zanamivir was determined from parallel bronchoalveolar lavage (BAL) and serum samples in both healthy and ALI rats following IV and INH dosing. All zanamivir concentrations were analysed using HPLC-MS/MS.

Results: The absolute bioavailability of nebulised zanamivir was 1.91%. In ALI animals, both the rate and extent of systemic absorption were reduced compared to healthy animals. Following IV administration, zanamivir concentrations in BAL were 3.1-, 4.0-, and 5.0-fold higher in healthy rats than in the ALI group at 30, 60, and 240 minutes post-dosing, respectively ($P \leq 0.05$). The $AUC_{30–240}$ in BAL after IV administration was approximately 3.3-fold higher in healthy animals (35,815 vs. 10,886 ng/mL×h). AUC comparison showed that BAL exposure after IV administration was 6.5-fold lower than after INH.

Conclusions: Our results confirm the advantage of INH administration in achieving therapeutic concentrations in the lungs and indicate that ALI significantly affects the permeability of zanamivir between the systemic circulation and lung tissue, which may influence treatment efficacy.

Keywords: inhalation, zanamivir, acute lung injury, bioavailability

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AUTOMATED BLOOD SAMPLING IN RATS - FIRST EXPERIENCES

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Background: Automated blood sampling in laboratory animals is crucial for improving research accuracy and reducing animal stress. The Automated Blood Sampler (ABS) is designed to streamline this process, offering precise and automated collection of blood samples.

Material/Methods: The ABS device integrates advanced sensors and algorithms to ensure accurate and consistent blood sample collection. It consists of high precision peristaltic pumps, an in-built fraction collector, a removable carousel, and in-line sensors that can differentiate between pure blood, saline solutions, and mixtures. Studies were conducted using different compounds and animal models to evaluate the device's performance.

Results: The ABS demonstrated high sampling success rates in multiple 24h pharmacokinetic studies: 80.89% in the THCP study, 83.95% in the CBD study, and 81.64% in the HHC study. The device significantly reduced animal stress, improved sample accuracy, and increased efficiency compared to manual blood sampling methods.

Conclusions: The ABS offers significant advantages over manual blood sampling, including reduced animal stress, improved accuracy, and increased efficiency. It has the potential to enhance the quality of research and ease laboratory procedures, enabling more stable and reliable data collection.

Keywords: Automated blood sampler, sample accuracy, laboratory efficiency

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DRUG TARGETING INTO THE INTESTINAL LYMPH

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Background: Intestinal lymphatic system is a large and important reservoir of immune cells. Drug targeting into the intestinal lymph could therefore be a viable strategy for modulating the function of the immune system, including the inflammatory response.
Material/Methods: Cannabidiol (CBD) was used as a model drug with known immunomodulatory effects. The extent of CBD absorption into the mesenteric lymph was measured using an anesthetized lymph duct-cannulated rat model and an awake thoracic duct-cannulated pig model. CBD was administered in the form of a basic oil solution and a surfactant-based nanoemulsion.
Results: The mean±SD CBD bioavailability via lymph was 2.0±0.9% for the nanoemulsion and 2.6±1.0% for the oil solution in rats (n=6, difference not significant). However, the nanoemulsion did increase the direct absorption of CBD into portal blood (21±9% vs. 1.9±0.9, $p<0.05$). Concordant results were found in pigs (n=3), where the mean±SD bioavailability via lymph did not differ significantly (0.35±0.35% for nanoemulsion vs. 1.23±0·63% for oil solution, p=0·46), whereas the direct bioavailability via portal blood was higher for the nanoemulsion (18.0±6·7% vs. 4·9±0·9%, p=0·04)

Conclusions: A large amount of CBD is transported through the intestinal lymph in both rats and pigs. CBD administration in the form of a nanoemulsion increases direct absorption into the blood while the lymphatic transport is largely unaffected.

Keywords: cannabidiol, bioavailability, lymphatic transport, lymph duct cannulation, rat, pig

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EFFECTS OF CANNABIGEROL ON CIA MODEL

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Background: This exploratory *in vivo* study aims to assess the anti-inflammatory properties of cannabigerol (CBG) in the collagen-induced arthritis (CIA) rat model of rheumatoid arthritis and to identify the molecular cascades involved.

Methods: Rats were randomized into four groups: placebo (PCB) – p.o. treated with 1 mL of 0.9% saline once daily, CBG – p.o. treated with 30 mg of CBD/day, glucocorticoids (GC) – p.o. treated with methylprednisolone 0.5 mg/kg/day, and negative control (CO) – p.o. treated with 1 mL of 0.9% saline once daily. CIA was induced in the PCB, GC, and CBG groups. Weight, arthritis score and width of paws were scored during the experiment. On day 29, rats were sacrificed and blood and synovial membrane samples were collected for ELISA, RT-PCR and Western blot analyses.

Results: Clinical scores showed significant improvement in the CBG vs. PCB on day 29 and in the GC vs. PCB on days 24, 27, and 29. MMP-3 levels in serum were significantly reduced in the GC vs. PCB. According to genes expression results from blood and synovial membrane, CBG acts notably through the downregulation of molecules such as TLRs, systemic NF-κB p65, STAT-3, and inflammasome-related components including NLRP1A, NLRP3, AIM2, gasdermin D, and caspase-1. On the protein level, no significant differences in TNF-α, IL-6, or MMP-3 were observed in the CBG vs. PCB, while the GC showed significant reduction of TNF-α and a trend toward decreased IL-6 and MMP-3 in synovial tissue.

Conclusions: Our findings indicate that CBG modulates distinct components of the inflammatory signaling pathways, but its effects were not sufficiently potent to produce significant clinical improvements in this model.

Keywords: cannabinoids, cannabigerol, rheumatoid arthritis, CIA model

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N-ACETYLCYSTEINE AND ITS THERAPEUTIC POTENTIAL IN AN ANIMAL MODEL OF ALLERGIC ASTHMA

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Background: N-acetylcysteine (NAC) is a classical mucolytic agent that, in addition to its mucolytic activity, also exhibits antioxidant activity. This could be beneficial in treating chronic inflammatory airway diseases, including asthma. We evaluated the ability of NAC to modulate airway defence mechanisms and inflammation after 10 days of administration (20 mg/kg/day and 60 mg/kg/day) in an experimental guinea pig model of allergic inflammation.

Material/Methods: The concentrations of inflammatory cytokines IL-4, IL-5, IL-10, IL-12, IL-13, GM-CSF, IFN-γ, and TNF-α were measured in BALF using a multiplex detection method. The concentration of remodelling marker TGF-β1 was measured in lung homogenates using enzyme-linked immunosorbent assay (ELISA). *In vivo,* changes in specific airway resistance and tracheal contraction amplitude were determined to evaluate the bronchodilator effect. The sensitivity of the chemically induced cough reflex was determined by an *in vivo* method. Ciliary beat frequency, assessed on washed tracheal cells, indicated the mucociliary clearance rate.

Results: Our data show that 10-day NAC administration led to a significant decrease in the regulatory cytokines IL-4, IL-5, and GM-CSF, which promote IL-1β secretion by blocking antioxidant responses. NAC reduced the number of chemically induced cough reflexes and ciliary beat frequency. NAC did not affect airway hyperreactivity parameters, namely specific airway resistance and tracheal smooth muscle reactivity.

Conclusions: In summary, we can state that NAC is a multifactorial drug, and in our experimental conditions of allergic inflammation, it showed positive effects on the levels of regulatory cytokines, which led to a reduction in the intensity of airway defence mechanisms.

Keywords: N-acetylcysteine, allergic asthma, allergic airway inflammation, allergic airway hyperresponsiveness

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THE ROLE OF BILIVERDIN REDUCTASE IN CANCER CELLS

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Background: Biliverdin reductase A (BLVRA) is a key enzyme in bilirubin metabolism, where it reduces biliverdin to bilirubin. Bilirubin is a potent antioxidant that protects cells from oxidative stress. Therefore, reduced or deregulated BLVRA activity may contribute to increased oxidative DNA damage, which is one of the factors leading to the neoplastic transformation of cells. There is evidence that BLVRA has also signaling functions, influencing various pathways involved in cell growth and survival. Deregulation of this signaling may affect processes such as apoptosis, proliferation, and differentiation, which are critical aspects of tumor development and progression. BLVRA expression may also differ between normal and tumor tissues, making it a potential biomarker for the diagnosis or prognosis of certain cancer types. At the same time, it may serve as a target for novel therapeutic strategies aimed at modulating its activity to regulate tumor growth.

Material/Methods: Human ovarian adenocarcinoma cell line A2780 was utilised as a reference cell line. Two specific cell clones (J and F) of A2780 overexpressed with BLVRA were selected as models for determining the role of BLVRA in the response of A2780 cells to different types of chemotherapy. Our study employed a range of analytical techniques, including western blotting, MTS assay and comparative proteomic analysis.

Results: After successful transfection of A2780 cells with an expression vector containing the BLVRA gene, we selected the two most significantly overexpressed clones J and F. Subsequently, the selected clones were tested for their response to various types of chemotherapy. The different responses of the clones to therapy compared to the control as well as the different proteomic profile indicating changes in heme metabolism and p53 signaling are currently the subject of our studies.

Conclusion: Investigating the role of BLVRA in relation to cancer appears to be highly important in order to better understand its involvement in tumor pathogenesis and to identify potential therapeutic interventions.

Keywords: A2780, BLVRA, overexpression, chemotherapy

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TIME COURSE OF GENE EXPRESSION OF 48 GENES OVER 48 HOURS IN PERIPHERAL BLOOD MONONUCLEAR CELL CULTURES AFTER STIMULATION WITH LIPOPOLYSACCHARIDE USING A NOVEL COMPLEMENTARY DNA SYNTHESIS METHOD

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Background: Efficient, cost-effective processing of RNA samples from 96-well plates is essential for high-throughput applications like drug screening. We aimed to develop a simplified, low-cost method for mRNA analysis in PBMC cultures and use it to monitor the time course of multiple genes expression following LPS stimulation.

Methods: PBMCs were isolated from the blood of healthy donors and seeded at 10^6 cells/mL in 96-well plates. Cells were stimulated with LPS (100 ng/mL) and harvested at 2, 6, 12, 24, 48 h. cDNA was made by our Cell to cDNA method involving lysing cells with 100µL water solution of 0.5% SDS, 10mM DTT, 1mg/mL proteinase K, 1 hour incubation at 50 °C, followed by inactivation at 90°C for 5 minutes and neutralisation with 1:1 dilution by 20% Tween 20 solution followed by reverse transcription. For qPCR amplification custom designed probe-based assays were used. All qPCRs were run with no-reverse transcriptase control and negative control. Custom-designed probe-based assays were validated for their cDNA specificity and effectivity.

Results: Of 56 targeted genes, 48 were found to be cDNA specific (not amplifying genomic DNA). 5 genes (HCRTR1, HCRTR2, HCRT, MMP3, NGF) were not expressed, and 4 (IL17A, IL2, VCAM, TrkA) showed minimal expression. 3 housekeeping genes (TBP, YWHAZ, HPRT1) were stably expressed. The expression kinetics of all remaining genes were mapped over 48 hours, allowing selection of optimal sampling points for each gene.

Conclusions: We present a simple method for direct cDNA synthesis from cell lysates with drastically reduced cost. Using this approach, we successfully tracked the time course of expression for 48 genes over a 48-hour period following LPS stimulation. This makes it ideally suited for time-resolved studies in immunology and streamline drug screening applications.

Keywords: PBMC, RNA isolation, qPCR, cDNA synthesis, drug screening, LPS, inflammation

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TARGETING RENAL FIBROSIS: THE THERAPEUTIC PROMISE OF KYNURENIC ACID

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Background: Kidney injury of various etiologies induces inflammation, immune activation, and upregulation of transforming growth factor-β (TGF-β), a key mediator of fibrotic progression. However, effective therapeutic options for renal fibrosis remain limited. Kynurenine (KYN) and its metabolite kynurenic acid (KYNA) exhibit immunosuppressive properties and may modulate fibrotic responses. In this study, we investigated the effects of KYN and KYNA in both cellular and animal models of kidney fibrosis.
Material/Methods: Mouse fibroblasts (NIH/3T3) were stimulated with recombinant TGF-β1 (5 ng/ml) and cultured for 24, 48, or 72 hours. Cells were divided into six groups (n=6): control untreated cells (C), TGF-β1-stimulated cells (TGF), and TGF-β1-stimulated cells treated with either KYN (3 µmol/l; TK3 and 10 µmol/l; TK10) or KYNA (50 µmol/l; Tka50 and 150 µmol/l; Tka150). KYN and KYNA were added together with TGF-β1 at the beginning of each experiment and additionally at a 24-hour interval in the 48- and 72-hour experiments. Cells were harvested for Western blot analysis at each time point. Wistar rats were divided into four groups (n=10): sham-operated rats (SHAM), rats subjected to unilateral ureteral obstruction (UUO), and UUO rats treated with KYNA (100 mg/kg every 24 hours p.o.; KYNA100, and 200 mg/kg every 12 hours p.o.; KYNA400) for 7 days. On day 8, rats were sacrificed and kidneys were collected for Western blot and histological analyses.
Results: In cells, KYN and KYNA treatments attenuated the expression of TGF-β- fibrotic pathway proteins. The most significant decrease (in %) was found in the TKa150 group at all timepoints: 24h (pSMAD2 -31±7 vs. TGF, $p<0.05$), 48h (Collagen I -33±11; αSMA -76 ±14; pSMAD2 -59±21; pp38 -52±12; pERK/ERK -58±15; pJNK/JNK -69±13; pMEK/MEK -84±21; all vs. TGF, $p<0.05$) and 72h (Collagen I -67±11; Vimentin -59± 5; αSMA -85±20; pSMAD2 -70±38; pp38 -68±30; pJNK/JNK -52±28 all vs. TGF, $p<0.05$).

In animal model, we observed a significant reduction in fibrotic area (-45±6; vs. UUO; $p<0.05$), α-SMA-positive area (-22±5; vs. UUO; $p<0.05$) and the expression of the fibrotic cascade protein pp38 (-43±15; vs. UUO; $p=0.05$) in the KYNA100 group. The treatment also alleviated structural tubular damage, as reflected by decreased cortical (-14±5; vs. UUO $p<0.05$) and medullary (-17±7; vs. UUO; $p<0.05$) distal tubule dilatation. Moreover, in the KYNA400 group, we observed a marked decrease in Collagen III (-49±17; vs. UUO; $p<0.05$) and Vimentin expression (-32±14; vs. UUO; $p<0.05$) both key components of fibrotic tissue. KYNA also modulates oxidative stress markers, increasing MnSOD expression (+12 ± 4; vs UUO; $p<0.05$) and decreasing iNOS levels (-31±10; vs UUO; $p<0.05$).

Conclusions: Our study demonstrates, for the first time, an antifibrotic potential of KYN and particularly KYNA by reducing the expression of both fibrotic markers and proteins in *in vitro* and *in vivo* fibrotic models. In addition, KYNA exerts antioxidant effects, further supporting its therapeutic potential.

Keywords: renal fibrosis, kynurenic acid, unilateral ureteral obstruction

Acknowledgment: This project was supported by APVV-23-0399, VEGA 1/0121/2022, VEGA 1/0513/2024.

ANTI-SLAMF7 ANTIBODY, ELOTUZUMAB, DOES NOT INDUCE SIGNIFICANT ADCC AGAINST CHRONIC LYMPHOCYTIC LEUKEMIA B-CELLS

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Background: Signaling lymphocytic activation molecule (SLAM) family 7 (SLAMF7) receptor has been found on normal B cells as well as neoplastic B cells of patients with various B cell chronic lymphoproliferative disorders including chronic lymphocytic leukemia (CLL). In CLL, the expression of SLAMF7 on CLL B cells is downregulated in comparison with normal B cells. Several SLAMF receptors have been suggested as potential drug targets for the development of novel anti-CLL therapies.

Material/Methods: Here, were analyzed the ability of elotuzumab, a therapeutic anti-SLAMF7 antibody, to induce antibody-dependent cellular cytotoxicity (ADCC) in CLL B cells isolated from peripheral blood of 5 patients with CLL. ADCC was analyzed by a flow cytometry method using elotuzumab at 100 μg/ml as ADCC-inducer, peripheral blood mononuclear cells (PBMCs) isolated from healthy donors as effector cells (E), CFSE-stained CLL B cells as target cells (T) and 7-AAD staining to distinguish between live and dead target cells. The E:T ratio was 8:1.

Results: In the absence of elotuzumab and PBMCs, the proportion of dead cells in control samples was 6.70 ± 4.76 (mean ± SD). Four-hour incubation of target CLL B cells with elotuzumab alone, PBMCs alone, and elotuzumab together with PBMCs increased the proportion of dead cells to 7.70 ± 4.90, 8.98 ± 5.29, and 9.55 ± 6.28, respectively. However, these increases were small and statistically non-significant.

Conclusions: Our findings show that elotuzumab is not effective in inducing ADCC against CLL B cells.

Keywords: SLAMF7, chronic lymphocytic leukemia, elotuzumab, ADCC, flow cytometry

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EXPLORING DRUG SUSCEPTIBILITY AND TRANSMISSION DYNAMICS OF TUBERCULOSIS AMONG UKRAINIAN PATIENTS: INSIGHTS SINCE THE WAR BEGAN

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Introduction: As a consequence of the ongoing war in Ukraine, the prevalence of tuberculosis (TB) has significantly increased in nations with a substantial influx of war refugees. Furthermore, it has been demonstrated that a substantial number of these patients were infected with resistant TB strains. The purpose of this study is to identify mutations linked to antituberculosis drug resistance and perform a molecular epidemiology investigation to assess transmission dynamics.

Material and methods: The samples were donated by Ukrainian citizens (n = 146) residing in the Czech Republic and Slovakia. Whole-genome sequencing (WGS) was performed on all isolates and the data were processed using a variety of bioinformatic tools.

Results: Through the WGS approach, resistance to at least one drug was confirmed in 44 of all patients: 26,7% to isoniazid (INH); 21,2% to streptomycin (STM); 19,9% to ethambutol (ETM) a 18,5% to rifampicin (RIF). Among patients with both sensitive and resistant forms of TB, the Beijing *2.2.1* sub-lineage was the most prevalent, comprising 32,2%. The most frequent mutations linked to resistance to individual drugs were: *katG* S315T (INH); *embB* M306V (EMB); *rpsL* L34A (STM); *gyrA* A94G (FQ).

Conclusion: Although the situation in the Czech Republic and Slovakia remains stable, the ongoing migration from Ukraine is reflected in a rise in resistant TB strains. The WGS technique provides a sophisticated approach for tracking of emergence and distribution of drug-resistant TB, thereby contributing to improved management.

Keywords: tuberculosis, drug resistance, transmission

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FROM EXPERIMENTAL ASTHMA MODELS TO NOVEL THERAPIES

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Background: Allergic asthma is a heterogeneous chronic inflammatory airway disease characterized by bronchial hyperresponsiveness (BHR), excessive mucus secretion, airway remodeling, and neuronal sensitization. These processes are regulated by multiple molecular pathways, including Rho-kinase signaling (ROCK1, ROCK2), epithelial cytokines (IL-25, IL-33, TSLP), and Th2 cytokines. Ion channels and membrane receptors—particularly TRPV4, TMEM16A, TRPM4, and bitter taste receptors (TAS2Rs)—have emerged as promising therapeutic targets due to their involvement in these pathways, which enable the modulation of inflammation, neuronal excitability, and smooth muscle contractility.

Material/Methods: We employed *in vitro* and *in vivo* methods (guinea pig OVA-sensitized model) to study the pharmacological modulation of Rho-kinases and ion channels (TRPV4, TRPM4, KCa3.1, TMEM16A) using their selective inhibitors and/or antagonists. Additional experimental techniques included immunocytochemistry, calcium imaging, β-hexosaminidase assays, airway resistance measurements, mucociliary clearance analysis, and cytokine quantification by ELISA and multiplex assays.

Results: Rho-kinase inhibition produced significant anti-inflammatory and anti-remodeling effects, including reduced expression of EGFR, TGF-β, and collagen V. Ion channel modulation demonstrated compound-specific regulation of mast cell degranulation, intracellular calcium signaling, airway smooth muscle contraction, and reflex responses such as cough and bronchoconstriction. The TAS2R agonist AMG reduced mast cell activation and improved mucociliary function. Inhibition of TRPV4, KCa3.1 and TMEM16A mitigated airway hyperreactivity and inflammation, confirming their pathophysiological involvement.

Conclusions: Our findings highlight the complex interplay between Rho-kinase signaling and ion channel activity in asthma pathogenesis. Pharmacological targeting of these pathways may allow for the development of personalized therapies that address both inflammatory and neurogenic components of the disease. Preclinical asthma models reflecting distinct endotypes remain critical for translational research.

Keywords: asthma, ion channels, Rho-kinase, airway remodeling, inflammation

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INOSINE PRANOBEX: AN UNUSED LIFELINE IN THE FIGHT AGAINST COVID-19

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Background: The rapid onset of the COVID-19 pandemic triggered an intensive investigation of therapeutic options to manage the disease before targeted treatment could be developed. Inosine pranobex, an immunomodulatory agent with antiviral properties, indicated potential for reducing disease severity due to its pharmacodynamic characteristics, including immune response stimulation and inhibition of viral replication.

Material/Methods: The research study serving as the foundation for this presentation retrospectively evaluated the impact of inosine pranobex on COVID-19 related mortality using data from the Institute of Health Information and Statistics of the Czech Republic. Two time periods were examined: the first (2020–2021) covered more lethal variants (pre-Omicron), and the second (2022–2023) encompassed the period dominated by the less severe Omicron variant. Patients were paired based on comorbidities associated with increased COVID-19 mortality risk (identified through literature and data analysis), COVID-19 treatment, vaccination status, and post-infection immunity.

Results: The primary endpoint was defined as the case fatality rate assessed at three months following a confirmed COVID-19 diagnosis. Across both time periods, patients receiving inosine pranobex (administered within 0–5 days following diagnosis) exhibited a statistically significant therapeutic benefit, with untreated patients demonstrating a relative case fatality rate increase of circa 50 % compared to the treated cohort. These findings are consistent with previous studies, which uniformly report a significant benefit of inosine pranobex in the management of COVID-19.

Conclusions: Inosine pranobex merits further investigation for its therapeutic potential in the management of COVID-19, specifically for patients ineligible for targeted agents approved for its treatment, as well as an adjunctive therapy in patients receiving these agents. Finally, if a new pandemic were to emerge due to a novel viral pathogen lacking established therapeutic interventions, urgent research efforts should be directed toward investigating the potential efficacy of inosine pranobex for the management of such a viral disease.

Keywords: COVID-19, pandemic, inosine pranobex, mortality, risk factors

Acknowledgment: We express our gratitude to the Institute of Health Information and Statistics of the Czech Republic, particularly to Jiří Jarkovský and Ondřej Šanca, for providing the data.

TESTING BORON DERIVATIVES OF ANTI-ANDROGENIC DRUGS ON PROSTATE CANCER CELL LINES

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Background: Prostate cancer is one of the most common malignancies in men and its treatment involves androgen deprivation therapy. The aim of the study was to design and test new derivatives of already commonly used antiandrogen drugs, in which the nitro group was replaced by boronic acid, with potential for prostate cancer therapy.

Material/Methods: We synthesized a series of boron derivatives of nonsteroidal antiandrogens that were tested on androgen-dependent and androgen-independent prostate cancer cell lines. Non-carcinogenic cell lines were used as controls. *In vitro* toxicity was assessed using standard WST-1 assays, which allow quantification of cell viability based on metabolic activity. Cells were exposed to test compounds for 24 or 72 hours and IC_{50} values were determined by comparison with control drugs from the group of nonsteroidal antiandrogens.

Results: Several of the most active newly synthesized structural derivatives of nonsteroidal antiandrogens showed higher antiproliferative activity against androgen-dependent prostate cancer lines compared to the standards flutamide and bicalutamide, while at the same time having lower toxicity against non-carcinogenic lines.

Conclusions: Boron derivatives of antiandrogen drugs show promising antiproliferative effects on prostate cancer while exhibiting lower toxicity compared to standard drugs. These results support further research on boron compounds in the field of antiandrogen therapy.

Keywords: boron derivatives, antiandrogens, prostate cancer, *in vitro* testing

CARBON TETRACHLORIDE-INDUCED CHANGES IN ARTERIAL SEGMENT REACTIVITY AND THE EFFECT OF PYCNOGENOL TREATMENT FIGURED BY COMPUTATIONAL MODELING

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Background: Carbon tetrachloride (CCl_4) is a technical substance used mainly in the past, well known for its noxious effects to the liver, kidneys and brain as well as serious damage of endothelial layer of vessel walls. Pycnogenol is a standardized extract from Mediterranean pine *Pinus maritima* bark, exerting antioxidant, vasodilator and antiinflammatory properties. In our study investigating possible functional changes in the reactivity of isolated segments of the rat renal arteries, we set two basic aims: 1. to quantify contractile and relaxant responses of the segments of rat renal arteries by both basic approaches of current recording of vascular reactivity: classical descriptive evaluation and analysis of digital records of segment responses using computer-based modeling, 2. to assess possible changes of segment reactivity induced by treatment with carbon tetrachloride and pycnogenol.

Material/Methods: Adult male Wistar rats were divided into four groups: control (K), group treated by CCl_4 (C), group treated by pycnogenol (KP) and group treated by both CCl_4 and pycnogenol (CP). After 10 weeks of lasting treatment, we investigated the contractile responses of isolated and perfused vascular segments by a series of contractions induced by successively increasing bolus doses of noradrenaline (0.05; 0,1; 0,5; 1; 3; 6; 10 µg). After these series, relaxant responses were induced by a single bolus dose of acetylcholine (20 µg) in the state of segment precontraction.

Results: Descriptive evaluation of the contractile responses did not find out significant differences between groups at any of the doses of noradrenaline. We found an apparent decline in relaxant responses in both carbon tetrachloride groups compared to the control group, not recovered by pycnogenol. After evaluation by digital analysis, we revealed a significant difference in the vessel sensitivity at the dose 0,5 µg of noradrenaline between groups K and C, in the rate constant of relaxation at a dose of 1 µg of noradrenaline and model type 1-4 in both carbon tetrachloride groups compared to the control group. In addition, our results indicate a remarkable intersection of the vessel sensitivity curves of the control group and the carbon tetrachloride group not treated by pycnogenol, which we have not yet observed in any of our other models of pathological vascular reactivity.

Conclusions: Computer-based modeling greatly improves data extraction compared to descriptive methods and in our study it showed several significant differences in the characteristic parameters of vessel responses between groups, which determination is unavailable for traditional methods. Pycnogenol treatment did not recover the pathological vessel reactivity changes induced by carbon tetrachloride.

Keywords: vascular reactivity, contractile and relaxant responses, computer-based modeling, carbon tetrachloride, pycnogenol

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THERAPEUTIC POTENTIAL OF 1-METHOXYISOBRASSININ IN THE TREATMENT OF OVARIAN CANCER

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Background: Ovarian cancer, a leading cause of mortality among gynecological malignancies, is particularly challenging to treat in advanced, platinum-resistant stages. The identification of new therapeutic agents is critical. This study investigates the anticancer potential of 1-methoxyisobrassinin (MB-591), an indole phytoalexin derivative from Cruciferae plants, in cisplatin-sensitive (A2780) and cisplatin-resistant (A2780 cis) ovarian cancer cells.

Material/Methods: The antiproliferative effect of the studied compound was assessed using a colorimetric MTT assay. For cell cycle analysis, ROS production and mitochondrial membrane potential were measured using flow cytometry. The involvement of proteins in apoptosis and autophagy processes was detected by Western blot analysis.

Results: MB-591 exhibited antiproliferative effects in both cell lines, with greater sensitivity observed in A2780 cells. Treatment induced cell cycle arrest in the S and G2/M phases, altered regulatory protein expression, triggered intrinsic apoptosis pathways via caspase-9 activation and PARP cleavage, and caused DNA damage. MB-591 also elevated ROS levels and mitochondrial dysfunction. In addition to apoptosis, MB-591 also triggered autophagic responses, as indicated by changes in the expression and phosphorylation of key autophagy-related proteins, such as LC3A/B, ULK1, and PTEN in the cisplatin-resistant A2780 cis cells. Despite the induction of autophagy, this process was unable to prevent cell death, suggesting that autophagy may serve as a cellular survival mechanism in response to MB-591 treatment. NAC co-treatment mitigated MB-591-induced cytotoxicity, modulating cell cycle distribution, apoptosis, and inhibiting autophagy initiation.

Conclusions: MB-591 exhibits potent antiproliferative and pro-apoptotic activities in ovarian cancer cells, with differential mechanisms in resistant phenotypes. The compound specifically triggers autophagy in cisplatin-resistant cells, indicating its potential as a targeted therapeutic strategy. Its modulation by NAC highlights oxidative stress as a therapeutic target, supporting further development of MB-591 as a potential adjuvant in ovarian cancer treatment.

Keywords: antiproliferative, apoptosis, autophagy, indole phytoalexins, ovarian cancer

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POSTER PRESENTATIONS

ALTERATIONS IN LET-7 FAMILY MICRORNA LEVELS AS A POTENTIAL BIOMARKER FOR PULMONARY ARTERIAL HYPERTENSION

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Background: Pulmonary arterial hypertension (PAH) is a progressive and often fatal disease characterized by elevated pulmonary arterial pressure and vascular remodeling. In recent years, microRNAs (miRNAs) have emerged as promising biomarkers for a range of diseases, including PAH. Alterations in circulating miRNA levels in human plasma can potentially reflect underlying pathophysiological changes before clinical manifestations occur. In this study, we focused on the let-7 family of miRNAs, previously associated with cardiovascular diseases and known for their regulatory roles in cellular proliferation, differentiation, and inflammation. Based on prior evidence, we hypothesized that specific members of the let-7 family may exhibit altered plasma levels in patients with confirmed PAH and thus serve as potential early diagnostic markers.

Methods: A total of 39 patients underwent right heart catheterization at the National Institute of Cardiovascular Diseases (NUSCH) to confirm or exclude a diagnosis of PAH, where a sample of blood was obtained. The isolated miRNA from blood plasma was reverse transcribed into cDNA using TaqMan™ MicroRNA Reverse Transcription Kit, and quantitative PCR (qPCR) was performed using TaqMan™ MicroRNA Assays.

Results: The expression analysis did not reveal statistically significant differences in the plasma levels of any individual let-7 family member between PAH patients and those with other cardiovascular diseases. However, we observed suggestive trends in expression changes for certain miRNAs, with consistent, although not significant, upregulation or downregulation patterns among some PAH patients. The absence of statistical significance may be attributed to the considerable heterogeneity in the patient population.

Conclusion: Although our study did not demonstrate statistically significant changes in the expression of selected let-7 miRNAs between PAH and non-PAH patients, the observed trend-level differences suggest that members of the let-7 family may still hold promise as early biomarkers. These preliminary findings underscore the need for further research involving larger and more homogeneous patient cohorts to better evaluate the diagnostic potential of circulating miRNAs in PAH. Future studies should also explore longitudinal dynamics and functional correlations of miRNA expression with disease progression and treatment response.

Keywords: microRNA, PAH, qPCR, heart, let-7

Acknowledgement: This project was supported by APVV-23-0502

ANTIPROLIFERATIVE ACTIVITY OF PHYTOSPHINGOSINE ANALOGUES IN CANCER CELLS

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Background: Sphingolipid metabolites such as ceramides, sphingosine, sphingosine-1-phosphate, and phytosphingosine have emerged as key regulators of apoptosis. Previous studies have shown that phytosphingosine, a long-chain sphingoid base, effectively induces apoptosis in various cell types. Moreover, it was demonstrated that phytosphingosine, when combined with ionizing radiation, synergistically enhances the radiation response in radiation-resistant cancer cells. These findings highlight its potential as a radiosensitizing agent and underscore the importance of sphingolipid metabolism in cancer therapy. D-ribo-phytosphingosine, one of the most extensively distributed natural sphingolipid, has very similar structure to sphingosine. D-ribo-phytosphingosine activates protein kinase (MAPK)-mediated apoptosis. This project focuses on the synthesis of novel D-ribo-phytosphingosine analogues with various substituents. The goal is to enhance their potential antiproliferative activity against cancer cells. By identifying the most promising derivatives, this research aims to contribute to the development of clinically relevant compounds with potential applications in the treatment malignant deseases.

Material/Methods: The viability was assessed by MTT/MTS assays. the synthesized D-ribo-phytosphingosine derivatives were tested on various human cancer cell lines (breast carcinoma MCF7, colorectal carcinoma HCT 116, cervical carcinoma HeLa, leukemia Jurkat, melanoma BLM) and non cancer (BJ-5ta) to screen the cytotoxic effects of newly synthesized analogues and subsequent selection of the most effective analogues

Results: The preliminary evaluation study of the synthesised sphingomimetics, based on their ability to inhibit a proliferation of human cancer cells, showed promising cytotoxicity. The most effective componds were analogues with code name MZ2 and MZ3. The preliminary results revealed that HCl salts proved to be the most cytotoxic derivatives among all the tested substances, with IC_{50} values in the lower micromolar range on the Jurkat, HeLa and HCT-116 cell lines.

Conclusions: These results suggest the valid interest in structure and antiproliferative activitity relationship in searching for anticancer agent. Further studies are necesary to investigate the mechanism of action and to find out the relationship between structure, character and position of substituents and their antiproliferative activity.

Keywords: phytosphingosine, antiproliferative effect, cancer

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EVALUATION OF POTENTIATION OF ANTI-PD-L1 TREATMENT USING *IN VITRO* MODELS

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Background: Immunotherapy has become one of the most important approaches to cancer treatment. Inhibition of immune system checkpoints, specifically targeting programmed cell death protein and ligand (PD-1, PD-L1), is a key strategy in cancer immunotherapy. Anti-PD-L1 therapy, which blocks the interaction between PD-L1 on tumor cells and PD-1 on T-lymphocytes, can restore immune activity and promote tumor regression. Despite significant clinical success in several cancers, including non-small cell lung cancer and melanoma, the therapeutic efficacy of anti-PD-L1 therapy remains limited in a subgroup of patients. Therefore, potential strategies to enhance therapeutic response are currently under investigation. Our study focuses on evaluating the potentiation of anti-PD-L1 therapy in *in vitro* models.

Material/Methods: To study the potentiation of the antitumor effect of checkpoint inhibitors, a co-culture model of activated T-lymphocytes and a tumor cell line was designed. In the next phase of the study, the co-culture model was extended to include representatives of anti-PD-L1 therapy. Two human tumor cell lines expressing PD-L1 ligand, namely DU-145 and MCF-7, were selected for co-culture models. Salicylanilide derivatives were selected as test compounds according to the literature. Before setting up the model, these derivatives were tested for their ability to reduce the amount of PD-L1 in the two selected cancer cell lines by flow cytometry and Western blotting. Human peripheral blood mononuclear cells (PBMC) were used as the source of activated T-lymphocytes. In the co-culture model, CD3+ T-lymphocytes were stimulated with an anti-CD3 antibody and then co-cultured with a selected tumor cell line pre-treated with a selected salicylanilide. Then, the frequency of the CD3+/CD8+ T-lymphocyte subpopulation was determined by flow cytometry.

Results: Two types of co-culture models of activated T-lymphocytes and PD-L1-expressing tumor cell lines, namely DU-145 and MCF-7, were developed. Their functionality was verified using salicylanilide derivatives. The co-culture model with the DU-145 tumor cell line was expanded to include an anti-PD-L1 antitumor immunotherapy representative and could thus be used to further study the potentiation of anticancer immunotherapy. Salicylanilide derivatives demonstrated the ability to enhance the effect of T-lymphocytes against both tumor cell lines.

Conclusions: Our co-culture models are of great importance for the study of potentiation of antitumor immunotherapy and can thus contribute to the development of new drugs and their synergistic combination with checkpoint inhibitors.

Keywords: cancer, checkpoint inhibitor, anticancer immunotherapy, co-cultivation model

Acknowledgment: The design of the co-cultivation model and study of potentiation of anti-PD-L1 treatment was supported by the projects: MUNI/A/1424/2023 and MUNI/A/1494/2024.

ERYTHROPOIETIN RECEPTOR OVEREXPRESSION CAUSES PACLITAXEL RESISTANCE IN OVARIAN ADENOCARCINOMA A2780 CELLS

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Background: The presence of the erythropoietin receptor (EPOR) on the various types of cancer cells has been demonstrated to contribute to their inefficient response to standard pharmacotherapy. The fundamental impact of the EPOR is understood to correlate with various molecular pathways involved in the regulation of cell growth and proliferation, and consequently cancer progression. In addition, the association between the functionality of the EPOR and the cells' responsiveness to specific therapeutics has been demonstrated. The relationship between the EPOR overexpression in the A2780 cell line (human ovarian adenocarcinoma) and the development of resistance to paclitaxel is presented in the current study.

Material/Methods: In the present study, A2780 cells were utilised as a reference cell line. Three specific cell clones (C, T and V clones) of A2780 overexpressed with EPOR were selected as models for understanding the mechanism of chemoresistance to paclitaxel. The study employed a range of analytical techniques, including western blotting, MTS assay and comparative morphologic analysis.

Results: A comparative assay of the expressed EPOR in each individual cell clone was conducted using western blot analysis. This analysis revealed three distinct EPOR isotypes, each with a molecular weight of 28 kDa, 50 kDa and 68 kDa, respectively. The T clone exhibited just 68 kDa EPOR isotype. This specific cell clone demonstrated the most significant level of resistance to paclitaxel, as determined by MTS assay, compared to the parental cell line, the negative control and the other two clones (C and V). The alterations in morphological characteristics were also present, the most significantly in the T clone cancer cells.

Conclusions: As a matter of fact, the increased chemoresistance to paclitaxel and the morphological changes of the A2780-EPOR clones are strongly associated with the EPOR isotype expression. We foresee that gene expression profiling of EPOR overexpressed clones will reveal significant alterations in overall gene expression in EPOR compared to controls, suggesting the most likely mechanisms explaining EPOR clones' changed properties.

Keywords: erythropoietin receptor, overexpression, resistance, paclitaxel, A2780

Acknowledgment:

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EVALUATION OF PRO-ANGIOGENIC PROPERTIES OF POLYHYDROXYBUTYRATE/CHITOSAN SCAFFOLDS WITH AGAROSE/GELATIN GEL ADDITION

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Background: The chorioallantoic membrane (CAM) is an avian extraembryonic membrane commonly used as an experimental model to study angiogenesis and its inhibition in response to various tissues, cells, or soluble factors. The present study aims to evaluate the pro-angiogenic potential of a composite scaffold consisting of polyhydroxybutyrate/chitosan (PCHLY) and agarose/gelatin gel (AG), using an *ex ovo* quail CAM model.

Material/Methods: On embryonic day (ED) 6, sterilized PCHLY and AG-PCHLY scaffolds were applied on the CAM surface. After a period of 72 hours (ED9), the CAM-material complexes were excised along with a 2 mm surrounding CAM tissue for histological and immunohistochemical analyses. The angiogenic response was quantitatively assessed by measuring the vascular index, which reflects the difference in the number of blood vessels in the surrounding CAM area of the implants at the beginning of treatment (ED6) and 72 hours after scaffold implantation (ED9). The formation of blood vessels in the surrounding area of the PCHLY and AG-PCHLY scaffold and cell invasion into the implants were evaluated using the markers of endothelial cells and hemangioblasts (QH1), myofibroblasts (α-SMA), and the proliferative activity of the cells (PHH3) with immunohistochemical staining.

Results: The evaluation of *in vivo* angiogenic activity of the tested scaffolds demonstrated angiogenic potential in both materials. However, the vascular index evaluation confirmed the higher angiogenic response of AG-PCHLY (81.59% ± 3.42%) compared to PCHLY (74.12% ± 4.90%). Microscopic evaluation revealed the formation of new CAM villi and their incorporation into both scaffolds, indicating good biocompatibility. Furthermore, immunohistochemical analysis confirmed the occurrence of angiogenesis and blood vessel ingrowth, supported by immunomarkers for α-SMA, QH1, and PHH3.

Conclusions: *In vivo* testing showed the pro-angiogenic potential of the tested scaffolds, which predispose these scaffolds for utilization in the field of regenerative medicine. The acellular scaffolds represent a simple and relatively cheap biomaterial without the need for additives such as growth factors or bioactive molecules, which are often used to improve angiogenesis. These features predetermine the possible future utilization of these natural biopolymeric scaffolds, mainly in the area of cartilage and skin regenerative medicine.

Keywords: angiogenesis, chorioallantoic membrane, gel, polymeric scaffold, quail embryo

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VETERINARY OVER-THE-COUNTER MEDICINES IN THE WORLD

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Background: Over-the-counter veterinary drugs (OTC vet drugs) are medications for animals that can be purchased without the need for a veterinarian's prescription. These drugs are typically used for common, mild conditions or preventive care in animals such as pets and livestock. The conditions for placing these products on the market vary from country to country around the world. In Slovakia, nutritional supplements for animals are registered and controlled through the State Veterinary and Food Administration of the Slovak Republic. **Material/Methods:** The aim of the study was to monitor the growth trend of global OTC vet drugs markets using available statistical data.

Results: The **OTC vet drugs** market is expected to expand its roots at a steady CAGR of 7.9%. The market is likely to hold a revenue of US$ 8.8 billion in 2023 while it is anticipated to cross a value of US$ 19.01 billion by 2033. The reason of increased sales are higher animal adoption, no need for a prescription, and easy availability and the extended list of drugs used on animals and humans. The United States **OTC vet drugs** market leads in terms of market share in the North American region. Though the United States market held a market share of 25.6% in the global market. The flourishing pet care industry, along with a rising number of online pharmacy platforms, is garnering the demand for OTC vet drugs. Europe is expected to be the second largest market, followed by the Asia Pacific region (APAC). The APAC region is expected to witness significant growth due to the increasing demand for pet care products and the growing number of pet owners in countries such as China and India. The largest share of the European OTC vet drug market is accounted for by Germany.

Conclusions: Globally, the market is influenced by various trends that shape its dynamics and drive its growth, which include: increasing emphasis on preventive care, growing demand for natural and herbal OTC pet medications, technological advancements in medication delivery and the growth of e-commerce and online retail.

Keywords: OTC vet drugs, OTC vet drugs market

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PHARMACOGENETICS OF COMT AND HOMOCYSTEINE METABOLISM IN PARKINSON'S DISEASE: IMPLICATIONS FOR THERAPEUTICS

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Background: Parkinson's disease (PD) is a progressive neurological disorder characterized by motor and non-motor symptoms, with its aetiology involving a combination of genetic and environmental factors. The catechol-O-methyltransferase (COMT) gene, which provides instructions for an enzyme metabolizing catecholamine neurotransmitters like dopamine, plays a significant role in PD. Genetic variations in the COMT gene, including polymorphisms like Val158Met (rs4680), can influence enzyme activity and dopamine levels. Homocysteine, a biochemical marker, has been implicated in neurological disorders, with elevated levels posing a risk factor. This study explores the relationship between COMT gene polymorphisms and homocysteine levels in PD patients.

Material/Methods: Five COMT single nucleotide polymorphisms (SNPs) (rs2075507, rs4633, rs4818, rs4680, and rs165599) were analyzed to determine their association with levels of homocysteine, vitamin B12, active vitamin B12 (holotranscobalamin), vitamin D, and folic acid in 38 patients with PD and 30 healthy controls.

Results: Significantly elevated homocysteine levels were found in patients with PD compared to the control group. Furthermore, heterozygosity for the rs4633, rs4818, and rs4680 SNPs was associated with increased homocysteine levels.

Conclusions: COMT genetic variations, specifically heterozygous genotypes of the rs4633, rs4818, and rs4680 SNPs, may play a role in influencing homocysteine metabolism in patients with PD. These findings highlight the potential value of genotyping in assessing homocysteine levels in PD and suggest that COMT polymorphisms could be a factor in the variability of homocysteine levels observed in PD patients. Further research is warranted to fully elucidate the complex interplay between COMT genetics, homocysteine metabolism, and the pathogenesis and progression of Parkinson's disease.

Keywords: Parkinson's Disease, COMT gene, homocysteine, polymorphism,

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EFFECT OF SCARB2 ON THE RESISTANCE OF OVARIAN ADENOCARCINOMA CELLS TO CHEMOTHERAPY

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Background: Ovarian tumours are one of the most serious gynaecological malignancies. Sometimes, and especially in the case of recurrent conditions, so-called drug resistance occurs. Mechanism of drug resistance is characterized by gene expression changes that unable drug-induced cell cycle arrest and cell death. SCARB2 (Scavenger Receptor Class B Member 2) also known as Lysosomal integral membrane protein 2 (LIMP-2) is a glycoprotein that is located primarily in membranes of lysosomes and endosomes. There has been nearly no evidence of SCARB2 function in chemotherapeutic resistance in ovarian cancer.

Material/Methods: We used ovarian adenocarcinoma cell line A2780 (negative control) and its clones H, L V with overexpressed SCARB2 gene as our biological models. To analyze the effect of SCARB2 overexpression on chemotherapeutic resistance of A2780 cells, we used western blot analysis, MTS assay and comparative proteomic analysis.

Results: Comparison of metabolic activity of the parental and SCARB2 overexpressed cell clones after exposure to cisplatin, carboplatin, topotecan and paclitaxel (for 72 hours) showed significant changes in IC_{50} concentration. Clone V demonstrated the most significant drop in IC_{50} value in all tested chemotherapies. Also, clone L showed higher sensitivity to the tested drugs. Comparative proteomic analysis revealed some significantly upregulated and downregulated proteins. The most upregulated ones were involved in the signalling pathways associated with the lysosome. The protein expression was also validated using western blot analysis.

Conclusions: Our work suggests that there is a lot of unknown about the function of SCARB2 in the field of drug resistance, that are worthy of deeper studying.

Keywords: A2780, ovarian cancer, chemotherapy, clone

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ANTIBIOTIC RESISTANCE PROFILES OF CLINICAL ISOLATES OF COAGULASE-POSITIVE AND COAGULASE-NEGATIVE *STAPHYLOCOCCUS* SPP.

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Background: Staphylococci are common commensal bacteria found on human and animal skin and mucous membranes. However, pathogenic strains are major causes of hospital- and community-acquired infections. Their antibiotic resistance complicates treatment and increases the risk of failure. S. aureus is therefore classified among the ESKAPE pathogens—bacteria known for evading the effects of standard antibiotics.

Material/Methods: Samples (n = 31) were obtained from animal as well as human patients. Isolates were processed and cultured using standard culture methods for 24 hours at 37 °C. Primary isolates identification included Gram staining and the commercial biochemical STAPHYtest 24. The genus *Staphylococcus* was also confirmed by multiplex PCR targeting a segment of the *16S rRNA* gene; *S aureus* by primers for the *eap* and *nuc* genes. Genomic DNA used in mPCR was extracted using a specific isolation kit. DNA concentration was determined spectrophotometrically. Visualization was performed under UV light by gel electrophoresis. To determine the antibiotic susceptibility of isolates, a qualitative Kirby-Bauer method was chosen. Impregnated antibiotic discs (oxacillin 1 µg, cefoxitin 30 µg, doxycycline 30 µg, gentamicin 10 µg, erythromycin 15 µg, clindamycin 10 µg, ciprofloxacin 5 µg, and co-trimoxazole 25 µg were applied to Mueller-Hinton agar inoculated with a 24-hour bacterial suspension. After incubation, the isolates were divided into susceptible, intermediate, and resistant based on measuring the size of inhibition zones and compared to CLSI/FDA standards.

Results: Of the 31 samples, four samples were negative by mPCR, and 27 samples were positive (87%) for staphylococci. Of these, ten were confirmed as *S. aureus* (37%). Antibiotic susceptibility was as follows: All isolates were resistant to oxacillin and were mostly resistant (37%) and intermediate 40.7%) to erythromycin. The best susceptibility was demonstrated to cefoxitin, to which all isolates were sensitive. Among the studied strains, seven were evaluated as multiresistant (26%; resistant to at least three antibiotics from different pharmacotherapeutic groups) and one as extremely resistant (3.7%; resistant to at least six antibiotics from different pharmacotherapeutic groups).

Conclusions: The excessive and inappropriate use of antibiotics in human and veterinary medicine, which is subsequently transferred to the food sector, contributes to the selection of resistant strains and their subsequent spread between different host species and the environment. Investigation of the susceptibility of staphylococcal isolates provides information on the dynamics of resistance, and the occurrence of multi-resistant strains and can contribute to the optimization of antimicrobial therapy and infection control strategies within the One Health concept.

Keywords: *Staphylococcus*, antibiotics, resistance

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IMPACT OF PLATELET CONTAMINATION IN PBMC CULTURES: LITERATURE REVIEW AND OPTIMIZATION OF DENSITY GRADIENT ISOLATION

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Background: Peripheral blood mononuclear cells (PBMCs) are a heterogeneous population of immune cells consisting mainly of lymphocytes, monocytes, and a small proportion of dendritic cells. They are widely used in preclinical research to assess drug toxicity and to study immunomodulatory properties. For accurate immune assay interpretation, contamination with other blood components must be minimized. Platelets are frequently overlooked despite growing evidence of their immunomodulatory roles, both pro-inflammatory and anti-inflammatory.

Material/Methods: This study includes a brief literature review summarizing current knowledge on the role of platelets in immune regulation and their potential impact on PBMC-based assays. The second part is a methodological comparison of various centrifugation conditions and procedural modifications for Ficoll-Paque-based PBMC isolation. The aim was to minimize platelet contamination while maintaining PBMC yield.

Results: The optimized protocol included the standard three steps - 970g/20min Ficoll separation, followed by two washes (340g/13min and 200g/10min) - with one additional wash (200g/10min). For better comparability, results were normalized to ml of blood. The PBMC yield was 0.99×10^6/ml with 1.78×10^6/ml platelet contamination. In comparison, the standard protocol (without the extra wash) yielded 1.42×10^6/ml PBMCs with 6.73×10^6/ml platelets.

Conclusions: An additional 200g washing step significantly reduced platelet contamination in PBMC isolates with only a moderate reduction in cell yield. This protocol can improve reproducibility and interpretation of downstream immunological assays.

Keywords: PBMC, platelets, lymphocytes, Ficoll-Paque, isolation

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CLASTOGENIC EFFECT OF MICONAZOLE ON CHROMOSOMES OF CATTLE AND PIGS

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Background: Miconazole is an azole antifungal agent. It is a synthetic imidazole derivative with rapid fungicidal activity against yeasts and dermatophytes. In veterinary medicine, it is used for the local treatment of dermatophytosis in companion and farm animals. The genotoxic effects of miconazole are relatively poorly studied; it is known that miconazole induced a dose-dependent statistically significant increase in chromosome aberrations in the bone marrow and primary spermatocytes of mice. Our work aimed to examine the effect of selected concentrations of the fungicide miconazole (2.5 $\mu g.ml^{-1}$; 5.0 $\mu g.ml^{-1}$; 10 $\mu g.ml^{-1}$) on the genetic material of cultured peripheral blood cells of cattle and pigs using an *in vitro* chromosome aberration test.

Material/Methods: Blood was sterilely collected from healthy donors and subsequently cultured in RPMI 1640 medium (Sigma-Aldrich, USA) using a standard protocol. 24 h before the end of the culture, miconazole (CAS 22832-87-7) was added to the cell cultures in doses of 2.5 $\mu g.ml-^{1}$; 5 $\mu g.ml^{-1}$ and 10 $\mu g.ml^{-1}$. Colchicine was added 90 min. before the end of the culture and allowed the arrest of cell division at the metaphase stage. Metaphases (100 metaphases/concentration) were evaluated for the presence of chromatid (CB) - chromosome breaks (IB) and exchanges (CE, IE) using a light microscope (Nikon). The Chi-square test statistically processed the calculated percentages of induced breaks.

Results: Each of the three applied concentrations caused an increase in the percentage of breaks in lymphocytes of both animal species compared to the negative control. However, a statistically significant increase in chromosome aberrations ($p<0.05$) was only observed at a concentration of 10 $\mu g.ml^{-1}$ in cultured cattle lymphocytes and similarly in pig cells.

Conclusions: Genotoxicity testing helps assess the potential risks of various chemicals, including pharmaceuticals. This is beneficial not only for animal health and welfare but also for human health. In the metaphases of bovine and porcine lymphocytes, we noted a clastogenic effect of miconazole in the form of a statistically significant increase in the percentage of chromosome breaks after its treatment at a dose of 10 $\mu g.ml^{-1}$ ($p<0.05$).

Keywords: miconazole, genotoxicity, lymphocytes, cattle, sheep

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EFFICACY OF AN INNOVATIVE THERAPY IN PATIENTS WITH CYSTIC FIBROSIS

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Background: Cystic fibrosis (CF) is the most common, rare, genetically determined, autosomal recessively inherited and life-shortening disease. The presence of two mutations in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene, resulting in a defective CFTR protein, is responsible for the disease phenotype. Mutations in the CFTR protein lead to the accumulation of chloride ions along with water molecules inside epithelial cells, resulting in inadequate hydration of extracellular mucus and secretions. The clinical picture of the disease is characterized by pancreatic insufficiency, increased sweat salts and chronic inflammation of the bronchopulmonary system. The most important current advancement and trend in the treatment of CF is the use of causal therapy via CFTR modulators. The aim of this study was to investigate the efficacy of an innovative combination therapy with the CFTR protein modulators ivacaftor/tezakaftor/elexafactor (Kaftrio) in combination with ivacaftor (Kalydeco), and ivacaftor/lumacaftor (Orkambi).

Material/Methods: Data were collected via a patient questionnaire from December 2023 to February 2024. Twenty-six patients attending the CF centre in Banská Bystrica were included in the study. All patients had a confirmed F508del CFTR protein mutation and were indicated for CFTR modulator therapy. Patients were taking ivacaftor/ tezacaftor/ elexafactor in combination with ivacaftor and combination ivacaftor/lumacaftor. The efficacy of therapy was evaluated by statistically assessed parameters such as FEV1 (expiratory volume of air in one second), sweat chloride concentration and weight change before and after innovative therapy by one-way ANOVA paired t-test.

Results: 26 respondents participated in the survey and the average age of the respondents was 27.5 years. The majority of patients (92%) were on ivacaftor/tezacaftor/elexafactor combination in combination with ivacaftor for both 24 months and 36 months. Two patients (8%) were on ivacaftor/lumacaftor for 24 months and 36 months. The results showed that in 100% of respondents, the innovative therapy caused an increase in the FEV1 parameter, thereby improving functional lung capacity. Specifically, in 8 patients, the mean FEV1 values were 61.25% ± 30.93 before innovative therapy, which increased significantly to 73.25% ± 28.17 when modulators were used. Reduction in sweat chloride concentration occurred in 96% of the respondents. Specifically, 6 patients who took ivacaftor/tezacaftor/elexafactor in combination with ivacaftor experienced a significant decrease in mean chloride values from 104.58 ± 20.54 mmol/l to 47.66 ± 17.92. Up to 81% of the respondents reported weight gain. Specifically, a mean weight gain of 5.5 kg was noted in 8 patients.

Conclusions: Innovative therapy with CFTR protein modulators for CF patients proved to be effective and helped to improve the adverse consequences of the disease in combination with symptomatic therapy.

Keywords: CFTR, cystic fibrosis, ivacaftor, elexacaftor, tezacaftor

Acknowledgment: Thanks to Eva Bérešová, MD, PhD, from the CF Patient Centre in Banská Bystrica, for compiling the questionnaire and contacting respondents with innovative therapy.

EPICARDIAL ADIPOSE TISSUE VS. LEFT VENTRICLE: DIFFERENTIAL GENE EXPRESSION IN PATIENTS WITH ADVANCED HEART FAILURE

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Background: Epicardial adipose tissue (EAT), a metabolically active tissue surrounding the heart and major blood vessels, produces various bioactive substances like inflammatory adipokines, growth factors, and cardioprotective molecules. Its proximity to the myocardium implies it may play a part in cardiac health and disease through paracrine signalling that affects cardiac remodelling and hypertrophy. Our goal was to assess and compare the expression levels of several factors important for cardiac hypertrophy, development, and protection in both EAT and left ventricular (LV) myocardium in patients with end-stage heart failure. We also aimed to examine potential relationships between these factors within each tissue.

Material/Methods: EAT and LV tissue samples were obtained from 37 patients with end-stage heart failure undergoing heart transplantation. The study cohort included individuals with different types of cardiomyopathies and coronary artery disease. We measured gene expression levels using qRT-PCR and Western blot, concentrating on genes involved in cardiac development, hypertrophy, and inflammation.

Results: Most of the genes investigated showed higher expression in EAT than LV. Only VEGFA and adiponectin exhibited significantly lower expression in EAT. NF-kB p50 expression was similar in both tissues, while Connexin 43 was exclusively detected in the LV. We observed no differences in gene expression based on the cause of heart failure (cardiomyopathy or coronary artery disease) or the presence of diabetes mellitus. Significant correlations were found between genes related to cardiac hypertrophy (CALN/VEGFA, NFAT3/TGF-β1, NFAT3/ET-1) and between NF-kB and Caveolin-1 in both tissue types, suggesting intricate regulatory interactions.

Conclusions: Our primary finding is that EAT demonstrates greater activity than LV myocardium concerning several factors crucial for cardiac pathology. This underscores EAT's potential role as an active endocrine organ with complex interactions influencing the myocardium in the context of heart failure. Further research is needed to clarify the specific mechanisms and paracrine signalling pathways involved.

Keywords: Epicardial Adipose Tissue (EAT), Left Ventricle (LV), Gene Expression, End-Stage Heart Failure, Cardiac Pathology

Acknowledgment: The financial support for this research was provided by the APVV grant APVV-23-0557, „Signaling pathways of miRNA and ICD proteins in the human ischemic heart."

HIGH DOSES OF VITAMIN D IN TYPE 2 DIABETES PATIENTS

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Aim: This research aimed to comprehensively evaluate vitamin D supplementation in clinical practice in Slovakia, focusing on higher doses of cholecalciferol. The study investigated the effects of high doses of vitamin D on selected metabolic parameters in patients with type 2 diabetes mellitus (T2DM). The work includes a systematic literature review and original biomedical research focused on changes in PTH, HbA1c, Ca, P, the QoL and the safety and efficacy of high-dose vitamin D supplementation.

Methods: Our systematic literature review (according to PRISMA) included an analysis of 20 RCTs of 612 patients in the vitamin D group and 592 patients in the control group and focused on doses of vitamin D exceeding 4000 IU per day. In biomedical research, patients were divided into intervention and control groups. Monitored parameters included levels of 25(OH)D, PTH, HbA1c, Ca, P, and fasting blood glucose. Data on quality of life were collected using the SF-36 and DTSQ questionnaires. Statistical analysis included the Wilcoxon rank-sum, signed-rank, and Mann-Whitney U tests, while relationships between parameters were evaluated using Spearman's correlation analysis.

Results: The systematic review demonstrated that high-dose vitamin D supplementation significantly increased 25(OH)D levels (177.09%) compared to baseline values without recorded toxic levels. The average decrease in HbA1c was statistically significant in some studies (1.26%). Supplementation also contributed to a modest reduction in blood pressure. In biomedical research, a statistically significant decrease in PTH levels was observed in the intervention group ($p = 0.0156$), while HbA1c, fasting blood glucose, Ca and P levels did not show statistically significant differences between the first and last visit. Correlation analysis revealed a significant negative correlation between vitamin D levels and PTH (Spearman's coefficient = -0.69052; $p=0.0044$). Results from QoL questionnaires indicated a high level of treatment satisfaction in both groups, with patients in the intervention group reporting slightly better subjective assessments of QoL. All patients found the sublingual form of vitamin D administration convenient (75%).

Conclusions: The results suggest that high-dose vitamin D supplementation is safe and effective. Although no significant impact on glycemic control and other metabolic parameters was observed, the positive influence on QoL and the high level of patient satisfaction indicate the potential of high-dose vitamin D as an adjunctive therapy in the management of T2DM. However, further extensive and long-term studies are required to confirm these findings.

Keywords: Type 2 diabetes mellitus, vitamin D, high doses, metabolic parameters, systematic review, biomedical research

BEHAVIOURAL AND PHARMACOLOGICAL EVALUATION OF THE PSILOCYBIN ANALOGUE BAEOCYSTIN IN WISTAR RATS.

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Background: Baeocystin is a naturally occurring tryptamine-based compound found in various psychoactive mushrooms, including several species of the *Psilocybe genus*. Due to its structural similarity to psilocybin, which has shown therapeutic potential in the treatment of psychiatric disorders, there is a growing interest in investigating whether baeocystin exhibits comparable effects. This study investigated the pharmacokinetic profile and acute behavioural effects of baeocystin in Wistar rats.

Material/Methods: Behavioural assessments, including locomotor activity and its spatial characteristics (in the open field test) and sensorimotor gating measured by prepulse inhibition were evaluated after subcutaneous administration of 1.25 or 5 mg/kg baeocystin. Pharmacokinetics and brain-serum ratios were analyzed after the 5 mg/kg sc. dose.

Results: Pharmacokinetics demonstrated that both baeocystin and its metabolite, norpsilocin, have a very limited ability to cross the blood-brain barrier. Consistent with the pharmacokinetic profile, baeocystin had no significant effects on locomotor activity, exploratory behaviour, anxiety-like responses, or sensorimotor gating at doses of either 1.25 or 5 mg/kg.

Conclusion: Our results suggest that baeocystin has minimal to no behavioural effects in rats, probably due to its poor permeability across the blood-brain barrier. This limited penetration may account for its negligible neurobiological and psychedelic activity.

Keywords: baeocystin, pharmacokinetics, open field, prepulse inhibition, Wistar rats

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GERANYLATED FLAVONOIDS FROM *PAULOWNIA TOMENTOSA* SUPPRESS CANCER CELL PROLIFERATION AND TRIGGER APOPTOSIS

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Background: The presence of secondary metabolites in *Paulownia tomentosa* (Thunb.) Steud. (Paulowniaceae), particularly geranylated flavonoids, has recently garnered significant attention in the search for new biologically active compounds. Although these naturally occurring substances are found in a limited number of plant families, they are noteworthy for their potential pharmacological properties. Recently, geranylated flavonoids have been extensively studied for their various biological activities, which include anti-inflammatory, antibacterial, antioxidant, and cytotoxic effects.

Material/Methods: The tested substances were isolated from *P. tomentosa* and identified at the Department of Natural Drugs, Faculty of Pharmacy, MU. *In vitro* analyses were performed on DU-145, MCF-7, and THP-1 cell lines. Initial WST-1 assays assessed the time-dependent effects on cancer cell metabolism. DU-145, showing the highest sensitivity, was selected for further testing, including cell cycle analysis, apoptotic cell detection (annexin V/PI), and caspase-3/7 activity via flow cytometry. Western blotting was used to assess cell cycle regulatory protein levels. Finally, mitochondrial function was evaluated using JC-1 and MitoSox probes to measure membrane potential ($\Delta\Psi m$) and mitochondrial superoxide levels, respectively.

Results: We observed an antiproliferative effect, most pronounced in DU-145 cells, followed by THP-1, with the weakest effect in MCF-7 cells. These findings were supported by cell cycle analysis, which revealed increased accumulation of DU-145 cells in G1/G0 phase, further confirmed by western blotting. Additionally, we detected an increased proportion of annexin V^+/PI^- and annexin V^+/PI^+ cells, indicating apoptosis, aligns with increased caspase-3 and -7 activity. Mitochondrial assessments showed a concentration-dependent depolarization of the mitochondrial membrane and increased production of mitochondrial superoxide. The most pronounced effects in terms of antiproliferative and pro-apoptotic activity were observed after the treatment by diplacon, which was further selected as a leading compound from a series of isolated geranylated flavonoids.

Conclusions: In this study, we showed that geranylated flavonoids from *Paulownia tomentosa* can effectively inhibit the growth of cancer cells, particularly the DU-145 prostate cancer cell line. The tested substances further induce apoptosis and changes in the mitochondrial activity of cancer cells.

Keywords: apoptosis, cell cycle, diplacon, geranylated flavonoids, mitochondrial dysfunction

Acknowledgment: The study was supported by the project MUNI/A/1488/2024.

EMBRYOTOXIC EFFECT OF ALCOHOL ON DEVELOPING AVIAN EMBRYO AS AN ALTERNATIVE MODEL OF FETAL ALCOHOL SYNDROME

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Background: Alcohol consumption during pregnancy can lead to lifelong consequences for the foetus in the form of FAS (Fetal Alcohol Syndrome Q86.0). In 2022, the National Health Information Centre recorded 186 mothers in Slovakia who used alcohol during pregnancy out of a total number of 52,782 births. The avian embryo allows determining the direct effects of ethanol at the beginning of pregnancy without the influence of maternal malnutrition, concurrent drug use, the formation of a toxic acetaldehyde, or impaired placental function. Due to these facts, we decided to observe the harmful effects of ethanol on the developing chicken embryo during the period of organogenesis.
Material/Methods: Chicken eggs were divided into two groups for a twenty-four-hour (24H) and a forty-eight-hour (48H) observation. Different concentrations of ethanol (5%, 10%, 15%, and 20%) were administered in each group. Sampling occurred on the 9th embryonic day. The survivability of the embryos, the morphological changes, the embryo's body weight, and the liver's weight were observed. The liver samples were prepared for histological examination following standard protocols (haematoxylin-eosin; cutting thickness: 7 µm).

Results: The experimental results indicate a significant relationship between increasing concentrations of ethanol, longer exposure, and the severity of malformations in the developing avian embryo. The overall average mortality in the 24H group was 7% (n=61), while the average mortality in the 48H group was 25.75% (n=58). Significant differences in the weight of the embryo were recorded at 10% ethanol concentration (24H $\bar{x}$=1.717 g; 48H $\bar{x}$=1.353 g). Longer exposure to ethanol had no significant effect on the weight of the embryo's liver (24H $\bar{x}$=31.57 mg; 48H $\bar{x}$=26.41 mg). Histological examination revealed hepatocytes whose cytoplasm contained large vacuoles, clusters of cubic cells with pale cytoplasm, or hepatocytes with vacuolated cytoplasm, and in some places, the parenchyma was severely damaged (20% ethanol 24H, 15%, and 20% ethanol 48H).

Conclusions: The results demonstrate that, especially after 48 hours of exposure to elevated concentrations of alcohol (10%, 15%, and 20%), there is an increase in mortality, a decrease in weight, and histological changes in the liver parenchyma of exposed chicken embryos. We can state a higher mortality rate as well as a lower birth weight even in human foetuses that have been exposed to acute alcohol consumption. It is imperative to note that no safe level of alcohol consumption during pregnancy has been established.

Keywords: embryotoxicity, FAS, ethanol, alternative model

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BENEFITS AND RISKS OF OPIOID THERAPY IN CANCER PAIN - A SEARCH FOR A POSSIBLE ALTERNATIVE

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Background: More than 10 million new cases of cancer are diagnosed worldwide each year. According to the World Health Organization, lung, prostate, and colorectal cancers are the most common in men, while breast, colorectal, and lung cancers are most prevalent in women. Tumor-related pain is often the first symptom that prompts patients to seek medical attention, ultimately leading to diagnosis. This pain significantly impacts quality of life and is associated with insomnia, fatigue, impaired daily functioning, depression, and social isolation. While non-pharmacological methods can aid in pain relief, symptomatic treatment—particularly opioid analgesics—remains the cornerstone. However, their use is associated with adverse effects, underscoring the need for safer alternatives.

Material/Methods: This study aimed to evaluate cancer pain treatment, its benefits and risks under local conditions, and compare it with possible alternatives. The experimental part of the research was based on prescription records from the public pharmacy at the University Hospital with Polyclinic in Prešov. Data were collected from January 2019 to June 2021, focusing on prescriptions for strong opioids dispensed for diagnoses C00–C96 (according to ICD-10).

Results: During the study period, 2034 prescriptions and 3116 packages of strong opioid medications were dispensed. Fentanyl was the most commonly prescribed opioid, and transdermal patches were the most frequent dosage form. The main advantages of opioid analgesics include rapid onset, sustained pain relief, absence of a ceiling effect, parenchymal safety, and no impact on coagulation or hematological parameters. Despite these benefits, challenges included unacceptable side effects, opioid-resistant pain types, tolerance development, opioid-induced hyperalgesia, and risks of abuse and dependence.

Conclusions: Strong opioid analgesics remain essential in managing cancer pain due to their efficacy and pharmacological advantages. However, the drawbacks highlight the urgent need for alternative therapies. Promising options include cannabinoids and vitamin C, though further robust clinical research is necessary to confirm their safety and effectiveness.

Keywords: cancer pain, pain management, symptomatic treatment, opioid treatment, treatment alternative

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CHALCONE 1C AS A MEDIATOR OF ROS-ASSOCIATED CELL DEATH IN OVARIAN CANCER

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Background: Ovarian cancer remains one of the deadliest gynecological malignancies, primarily due to the development of resistance against conventional therapies, supported by multiple molecular resistance mechanisms. Chalcones have emerged as promising anticancer agents, capable of modulating oxidative stress pathways in these cells.

Material/Methods: The antiproliferative effects of chalcone derivative 1C were investigated in cisplatin-sensitive (A2780) and cisplatin-resistant (A2780cis) ovarian cancer cell lines. Cytotoxicity was assessed by MTT assay. ROS production, cell cycle distribution, mitochondrial membrane potential, apoptosis induction, and the involvement of signaling pathways (Nrf2, Akt, Erk1/2, NF-κB) were analyzed using flow cytometry and Western blotting. The role of oxidative stress was further studied by co-treatment with N-acetylcysteine (NAC).

Results: Compound 1C induced significant cytotoxicity, G2/M phase cell cycle arrest, mitochondrial membrane potential loss, and apoptosis in both ovarian cancer cell lines. These effects were associated with elevated ROS production and modulation of signaling pathways, including downregulation of Akt and p-Erk1/2 and changes in Nrf2 expression, differing between sensitive and resistant cells. NAC significantly attenuated the effects of 1C, confirming the role of oxidative stress in the observed antiproliferative mechanisms. Moreover, 1C caused DNA damage evidenced by γ-H2A.X phosphorylation and modulated expression of cell cycle and apoptosis regulatory proteins p21, PCNA, Rb, Bad, PARP.

Conclusions: The chalcone derivative 1C exerts potent antiproliferative effects against both sensitive and resistant ovarian cancer cells through ROS-mediated mechanisms, suggesting its potential for overcoming drug resistance in ovarian cancer therapy

Keywords: ovarian cancer, chalcone, oxidative stress, apoptosis, cell cycle

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EFFECT OF SHORT-TERM EXPOSURE TO MICONAZOLE ON BOVINE LYMPHOCYTES *IN VITRO*

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Background: Miconazole belongs to the group of azole fungicides; its mode of action is based on inhibition of 14-α-demethylase, the enzyme needed for transformation of lanosterol to ergosterol. Inhibition of ergosterol formation leads to increased membrane permeability, loss of intracellular potassium, and accumulation of toxic peroxides. Miconazole is currently used topically and is intended for the treatment of candidiasis and dermatophytosis. The genotoxic effect of miconazole has been rarely documented. Chromosome aberrations induction was detected in dose dose-dependent manner in mouse bone marrow cells. The aim of our work was to analyse the effect of short-term exposure (4h) of bovine lymphocytes to miconazole through the induction of micronuclei and apoptotic changes.

Material/Methods: Blood was taken from two healthy donors (a 6-month-old bull, a crossbreed of Holstein cow) under sterile conditions and cultivated according to the OECD Micronucleus test *in vitro* (no. 487). The micronucleus test was analysed under the light microscope. Apoptotic changes were assessed by TUNEL (Terminal deoxynucleotidyl transferase dUTP nick end labelling; Roche Diagnostics, Germany) assay according to the manufacturer's instructions under the fluorescent microscope. Bovine lymphocytes were exposed to miconazole (CAS 22832-87-7; 1.25, 2.5, 5, 10, 25, and 50 $\mu g \cdot ml^{-1}$) dissolved in dimethylsulfoxide (DMSO; Sigma, USA) for the last 4 h of the cultivation process. Statistical analysis was performed by Chí square test and ANOVA+Dunnett post-test

Results: Statistically significant changes in MN induction were observed from concentration of 10 $\mu g \cdot ml^{-1}$ ($p<0.05$; $p<0.01$; $p<0.001$) in donor no. 1. In donor no. 2, significant changes were found at concentrations 25 ($p<0.01$) and 50 $\mu g \cdot ml^{-1}$ ($p<0.001$). The CBPI value decreased with increasing miconazole concentration; in donor no. 1 a significant change was observed from miconazole concentrations of 25 $\mu g \cdot ml^{-1}$ ($p<0.01$; $p<0.001$) and in donor no. 2 from a concentration of 10 $\mu g \cdot ml^{-1}$ ($p<0.05$; $p<0.01$; $p<0.001$). A statistically significant induction of TUNEL-positive cells in both donors was detected from a concentration of 5 $\mu g \cdot ml^{-1}$ ($p<0.05$; $p<0.01$; $p<0.001$) in a dose-dependent manner.

Conclusions: Results of our work show the potential genotoxic and cytotoxic effects of miconazole in bovine lymphocytes *in vitro*. Fungicide drugs (pesticides) often act on non-target organisms via environmental exposure, and therefore, there is a need to assess their effect.

Keywords: cytotoxicity, genotoxicity, lymphocytes, miconazole, micronucleus

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RESISTANCE TO TAMOXIFEN IN BREAST CANCER CARCINOMA: THE ROLE OF LYSOPHOSPHATIDYLINOSITOL DYNAMICS

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Background: Breast carcinoma is the most common cancer affecting women and is often treated with hormone therapies that target the estrogen receptor (ER). Despite these interventions, many patients eventually develop secondary resistance to hormonal treatment, and the underlying molecular mechanisms remain poorly understood. Furthermore, there is a lack of predictive molecular biomarkers to diagnose this secondary resistance. We aimed to determine whether alterations in lipid metabolism in Tam5R breast cancer cells contribute to the development of resistance to hormonal therapy. In particular, we have focused on the involvement of the extracellular signal-regulated kinases 1 and 2 (ERK1/2) pathway, the activation of which is linked to various human cancers, and can be triggered by lysophosphatidylinositol (LPI) through G protein-coupled receptor 55 (GPR55). We also seek to evaluate whether Tamoxifen-resistant cancer cells can generate lipid signaling molecules that drive resistance and to investigate the intracellular trafficking of these molecules. Overall, lipidomics profiling aimed to identify potential molecular biomarkers of Tamoxifen resistance among the products of dysregulated lipid metabolism.

Material/Methods: We utilized an LC-MS approach to analyze the lipidome of Tamoxifen-resistant breast cancer cell lines MCF7 and T47D (Tam5R). Secondary resistance to Tamoxifen was induced by prolonged cultivation with the drug in the media. We quantified phosphorylation status of ERK in WT and Tam5R cells in respect to changes of LPI levels or GPR55 activation. Confocal microscopy enabled us to observe the changes in the dynamics of the LPI precursor, exogenous phosphatidylinositol (PI), in the cells treated with a PI-fluorescent probe.

Results: The levels of LPI were increased in the Tam5R cells compared to WT cells. We demonstrated that Tam5R cell lines have higher p-ERK levels than WT cells. ERK1/2 phosphorylation in WT cells using exogenous LPI was upregulated, and the upregulated ERK1/2 phosphorylation in Tam5R cells was diminished by the GPR55 inhibitor.

Tam5R and WT cells exhibit different dynamics of exogenous PI. WT cells distribute the PI into the mitochondria and endoplasmic reticulum, while Tam5R cells allocate it only to the endoplasmic reticulum, which also displayed some morphological changes compared to the WT endoplasmic reticulum.

Conclusions: Changes in glycerophospholipid metabolism in Tamoxifen-resistant breast cancer cells might be the source of excessive LPI used to activate ERK1/2 signaling, which may represent an autocrine axis involved in the development of Tamoxifen resistance itself.

Keywords: breast carcinoma, tamoxifen resistance, lysophosphatidylinositol, GPR55

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A SYNTHETIC DERIVATIVE OF NATURAL SPIROBRASSININ INDUCES APOPTOSIS AND MODULATES SIGNALING PATHWAYS INVOLVED IN COLORECTAL CANCER CELL METASTASIS

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Background: Colorectal cancer (CRC) is among the most common malignant tumours and carries a high mortality rate. Epidemiological evidence that greater consumption of Brassicaceae vegetables—rich sources of indole phytoalexins—can reduce the risk of CRC and other gastrointestinal cancers has spurred further research in this area.

Material / Methods: The synthetic indole phytoalexin MB-653 was dissolved in DMSO (final concentration ≤ 0.2 %). Cytotoxicity was tested in HCT116 and Caco-2 colorectal carcinoma cells and non-malignant MCF-10A breast epithelial cells cultured in supplemented RPMI-1640 or DMEM/DMEM-F12. Cell viability (initially > 95 %) and proliferation were assessed after 72 h exposure to 1–100 µM MB-653 using the MTT assay to determine IC_{50} values. Cell-cycle distribution was analyzed at 24, 48, and 72 h by flow cytometry. Mitochondrial membrane potential was measured by TMRE staining, while apoptosis was evaluated by Annexin V/PI staining, caspase-3/7 activation assays, and acridine-orange/PI fluorescence microscopy. Expression of apoptosis-related proteins was assessed by Western blotting. All experiments were done in triplicate; flow cytometry data were analyzed using FlowJo v10.

Results: In our study we demonstrated that MB-653, a synthetic derivative of the natural indole phytoalexin spirobrassinin, suppresses the proliferation of colorectal cancer cells (IC_{50} = 5.8 ± 0.3 µmol L^{-1} for HCT116 cells and 6.1 ± 2.1 µmol L^{-1} for Caco-2 cells). The antiproliferative effect of MB-653 was associated with the induction of apoptosis—evidenced by activation of caspases-3 and -7, mitochondrial dysfunction, and an increased proportion of apoptotic cells on AO/PI staining-as well as with cell-cycle arrest in the G2/M phase in both cell lines.

MB-653 also modulated key signalling pathways linked to metastasis and epithelial–mesenchymal transition (EMT). Moreover, we confirmed its safety after both local and parenteral administration.

Conclusions: Taken together, these findings highlight the considerable therapeutic potential of MB-653 in the treatment of colorectal cancer.

Keywords: colorectal cancer, indole phytoalexin, EMT, apoptosis, metastasis

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EVALUATION OF THE ANTI-INFLAMMATORY ACTIVITY OF BROUSSOFLAVONOL B IN A MODEL OF INDUCED PLANTAR EDEMA IN LABORATORY MICE

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Background: Prenylated phenols are natural substances that are used in traditional medicine for their wide range of biological effects. Broussoflavonol B was isolated from *Broussonetia papyrifera* with various biological activities, such as antioxidant and anti-inflammatory activity. The anti-inflammatory activity of broussoflavonol B has been described *in vitro*, and inhibition of NF-κB and IL-1β activity has been demonstrated. The next step in the investigation of the biological activity of these natural compounds is to verify their anti-inflammatory potential *in vivo*. The experiment follows the previous *in vitro* research phases, which confirmed the anti-inflammatory activity of the selected natural substances.

Material/Methods: The evaluation was performed in a mouse model of induced plantar edema. An animal model is essential at this stage of the evaluation of the activity of the substances, as the complexity of the mammalian organism cannot be replaced by an alternative method with the same reliability and predictive value. Forty 6-week-old male laboratory mice divided into four groups were included in the experiment. Two groups were orally administered broussoflavonol B in two doses; indomethacin was used as a reference substance, and the control group was administered vehicle only. 0.1 ml of 1% carrageenan solution was applied intraplantarly to induce experimental edema. Changes in volume parameters were monitored plethysmometrically at defined intervals. At the end of the experiment, the animals were sacrificed by cervical dislocation, and sampling (planta) was performed for histopathological examination.

Results: In experimental groups, compared to the control group, a statistically significantly lower increase in paw volume caused by the current inflammation, comparable to the reference substance (indomethacin), was demonstrated at several time intervals.

Conclusions: The results achieved confirm, in the selected animal model, the expected antiphlogistic effect of broussoflavonol B, which was also shown by the histopathological examination of the collected tissue.

Keywords: plethysmometry, paw, *in vivo*, *Broussonetia papyrifera*

Acknowledgment: The work was supported by the GAČR grant Role of prenylation and glycosylation patterns in anti-inflammatory activity and metabolism of natural phenolic compounds (GA23-04655S)

EVALUATION OF THE ANTIMICROBIAL ACTIVITY OF GLUCOSE SOLUTIONS AFTER RADIATION TREATMENT USING AN *IN VITRO* METHOD

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Background: Contemporary research actively explores the potential of irradiation for modifying the properties of bioactive compounds. Ionizing radiation is regarded as a tool for targeted molecular changes, capable of initiating complex chemical rearrangements and creating new functional properties.

Materials and Methods: This study evaluates the effect of radiation treatment on the antimicrobial properties of glucose. To assess the biological activity of treated samples, the following microorganisms were used: *Bacillus subtilis* 090, *Lactobacillus acidophilus* and *Candida albicans*.

The experimental procedure included the irradiation of glucose solutions using the M-30 microtron. A radiation stand equipped with a Pu-α-Be source was also employed to provide combined γ- and neutron fluxes. The M-30 microtron utilized a 1 mm tantalum (Ta) plate to generate bremsstrahlung γ-radiation, and a special assembly with a 1 cm lead (Pb) plate, in addition to Ta, to produce photoneutron radiation. In the experiment, the fluence of the output beam of accelerated electrons, with an energy of 18.5 MeV, was recorded. Notably, the fluence reached 5×10^{14} eV/cm² and 5×10^{15} eV/cm² during γ- and combined γ/photoneutron irradiation, respectively. Samples were placed 30 cm from the M-30. The second irradiation setup included a blockhouse-type stand with "neutron-stop" moderators and a Pu-α-Be source (type IBN-VIII). The dose rate of γ and neutron radiation was 5.0×10^{8} Gy/sec, with a neutron flux of 2.7×10^{6} neutrons/sec. The neutron flux density was measured using a certified MKS-RM1401K radiometer.

Samples of irradiated and non-irradiated glucose in dry powder form were prepared for analysis. A suspension was created from daily bacterial cultures using the 0.5 McFarland turbidity standard (1.5×10^{8} CFU), determined via a Den-1 densitometer. Microbiological analysis was conducted using a sterile 96-well titration plate. The minimum inhibitory concentration was determined using serial dilution up to the 8th dilution. Exposure time was 1 hour in a thermostat at 36.8–37.0°C. The experiment followed the *in vitro* time-evolution method, with 48-hour intervals between stages.

Results: The study found that all tested glucose solutions exhibited varying biological activities toward *Bacillus subtilis* 090, *Lactobacillus acidophilus*, and *Candida albicans*.

Notably, for the probiotic spore-forming strain *Bacillus subtilis* 090, a stable stimulation of microbial growth was observed with all tested glucose solutions. This trend persisted throughout the entire observation period. In contrast, for the non-spore-forming probiotic *Lactobacillus acidophilus*, a stimulating effect was recorded at the first stage when using solutions irradiated with γ-rays (5×10^{14} – γ) and under exposure to the Pu-Be source. However, by the second stage, an inhibitory effect on microbial growth was observed in all test samples. Importantly, irradiated glucose solutions demonstrated a clearly pronounced antifungal effect against *Candida albicans* at both stages of the study, showing consistent inhibition throughout the observation period.

Conclusions: The findings confirm the potential and practicality of using radiation technologies to develop new biologically active substances with targeted effects.

Keywords: irradiation, antimicrobial activity, glucose, microtron M-30.

PRO-APOPTOTIC POTENTIAL OF PSEUDEVERNIA FURFURACEA (L.) ZOPF IN BREAST CANCER THERAPY

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Background: Apoptosis, or programmed cell death, is a fundamental target in cancer treatment strategies. Breast cancer (BC) is the most commonly diagnosed malignancy in women and includes several molecular subtypes such as estrogen receptor-positive (ER+), HER2-positive (HER2+), and triple-negative breast cancer (TNBC). The resistance to conventional chemotherapies in these subtypes highlights the need for novel therapeutic agents, including natural compounds that can selectively induce apoptosis.

Material/Methods: We investigated the pro-apoptotic effects of *Pseudevernia furfuracea (L.) Zopf* extract (PSE) and its isolated secondary metabolite physodic acid (PHY) in *in vitro* models of breast cancer subtypes (ER+, HER2+, triple-negative). Apoptosis induction was evaluated using flow cytometry, caspase activity assays, and Western blot analysis at 24, 48, and 72 hours.

Results: PSE and PHY treatment led to cell cycle arrest at the G1 checkpoint and activated the intrinsic apoptotic pathway. This was evidenced by altered expression of Bcl-2 family proteins, increased activity of caspases 3/7, cleavage of caspase 9, release of cytochrome c from mitochondria, and cleavage of PARP. These effects were time-dependent and observed consistently across all tested breast cancer cell lines.

Conclusions: PSE and PHY induced mitochondrial-mediated apoptosis in breast cancer cells through activation of the intrinsic pathway. These findings support the potential use of these natural compounds as pro-apoptotic agents in breast cancer treatment strategies.

Keywords: Pseudevernia furfuracea, physodic acid, breast cancer, apoptosis

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PHARMACOKINETICS, SYSTEMIC TOXICITY, AND ACUTE BEHAVIOURAL EFFECTS OF PHENETHYLAMINE DERIVATIVE 25E-NBOH IN RATS

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Background: 25E-NBOH is a novel psychoactive substance derived from phenethylamine, originally synthesized in 2010 for emission tomography. It has emerged as an alternative to classical psychedelics such as LSD, acting as a potent serotonin receptor agonist and reportedly inducing strong visual hallucinations.

Material/Methods: The study investigates the pharmacokinetics, systemic toxicity, and behavioural effects of 25E-NBOH in rats. Concentrations in brain and serum are measured via LC-MS/MS over 24 hours post-subcutaneous administration. Toxicity is assessed following OECD 423 guidelines. Behavioural effects are evaluated using the Open Field Test and Prepulse Inhibition of Acoustic Startle Response.

Results: Preliminary data suggest significant accumulation of 25E-NBOH in brain tissue, notable behavioural alterations, and systemic toxicity potentially leading to serotonin syndrome. Observations are compared with the effects of classical and other novel psychedelics (e.g., LSD, 25CN-NBOH).

Conclusions: 25E-NBOH presents potent psychoactive and toxicological effects in animal models. The findings support the inclusion of this compound in databases mapping the pharmacological profiles of psychoactive substances.

Keywords: 25E-NBOH, psychedelics, pharmacokinetics, toxicity, behavioural effects

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MITOCHONDRIA CRISTAE ARE NARROWED UPON CELLS´ GLUCOSE SENSING

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Background: An exact understanding of beta cells' glucose sensing on the molecular level is needed to target pharmaceuticals to cure pathologies, e.g., Diabetes mellitus type II. Mitochondria, via the elevated ATP synthesis and redox signaling, play an important role in glucose-stimulated insulin secretion (GSIS). Their shaping, including inner structure forming cristae, reflects the energetic state of the cell.

Material/Methods: INS1E cells, CRISPR/Cas9 technology, electron microscopy tomography FIB/SEM, transmission electron microscopy, 3D superresolution microscopy

Results: We observed that mitochondrial cristae became narrower in response to the enhanced ATP synthesis upon GSIS. The sharp edges of cristae lamellae have been considered for long time to be stabilized by rows of ATP-synthase dimers along the crista rims. Our observation of the bulky cristae with flat rims for rat insulinoma INS-1E cells incubated at low 3 mM glucose would then reflect disruption of the ordered ATP synthase dimers. To elucidate this, vestigial ATP-synthases lacking F_o-membrane subunit e or subunits e+g using CRISPR/Cas9 were studied in INS-1E cells. In control cells, prepared with scrambled sgRNA, electron microscopy tomography FIB/SEM, transmission electron microscopy, and 3D superresolution microscopy evidenced the decreased intracristal volume with cristae narrowing upon transition from low glucose (3 mM, non-stimulating insulin release) to saturated glucose concentrations (>11 mM), stimulating GSIS. Those changes were not observed upon e and e+g ablation. Surprisingly, cristae maintained the narrow morphology even at low glucose. This excluded the above hypothesis of sharp-edge stabilization by ATP dimeric rows. Instead of cristae being poerpendicular to the outer membrane, they were parallel to it, indicating disruption of MICOS complexes. Moreover, a fraction of the fragmented mitochondrial network formed rings. FIB/SEM detected fused criste inside these rings and also these inner rings were very narrow.

Conclusions: We conclude that the cristal membranes form a double membrane invaginated structure naturally, possibly also due to osmotic forces. Also, cells with ablated e and e+g possessed lower ATP-elevation responses to glucose, as well as GSIS kinetics were slightly or nearly completely impaired after e and e+g deletion, respectively.

Keywords: mitochondria, cristae, ATP-synthase, subunits g and e, CRISPR/Cas9

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MULTI TARGET-DIRECTED LIGANDS AS A NOVEL DRUGS

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Background: Drug design and synthesis in the context of neurodegenerative disease therapy are focused on the preparation of new drugs that could affect several biological systems simultaneously. Using this design, two or more pharmacophores are combined with each other within a single molecule, whereby this new molecule should have properties that are characteristic of both starting pharmacophores. These new multipotent compounds are referred to as "multi-target-directed ligands". Work deals with the synthesis of new derivatives that contain a carbamate and an aryloxyaminopropanol pharmacophore in one molecule. The presence of these groups in a single molecule makes them attractive in terms of possible Alzheimer's disease therapy in combination with their β-adrenolytic effect.

Material/Methods: The synthetic preparation of 32 new MTDL molecules was carried out by a three-/four-step synthesis. The prepared molecules contain carbamate and a modified aryloxyaminopropanol pharmacophore. Pharmacological tests were carried out on selected synthetic derivatives; their inhibitory effect on acetyl/butyrylcholinesterase (Ellman test on CaCo2 cell line, or pure enzyme), effect on β-adrenergic signalling (measurement of β-adrenergic activity via calcium signalling on a THP-1 cell line), their effect on the cell viability of HEK cell line was investigated (changes in cell adherence were monitored) and also their cytotoxicity (assays on endothelial cells isolated from rat brain, endothelial cell viability was assessed by quantification of adenosine triphosphate released from the cells using the ATP assay).

Results: For all synthesized molecules, their physico-chemical properties (melting point, retention factor, yield) as well as their chemical structure (using 1D and 2D NMR spectroscopy) were determined. Tested selected derivatives did not show anticholinesterase activity on CaCo2 cells. However, acetyl/butyrylcholinesterase inhibition was confirmed *in vitro* by the Ellman test using pure enzymes. Seven of the twelve tested derivatives demonstrated an effect on β-adrenergic signaling comparable to the atenolol. When monitoring the cytotoxicity of selected derivatives on HEK cells, it was found that it was necessary to reduce the concentration of the tested substances tenfold. In testing of cytotoxicity on primary endothelial cells, it was found that all seven tested derivatives showed minimal toxicity at concentrations IC_{50}<2 mM.

Conclusions: The obtained pharmacological tests show that the synthesized derivatives containing carbamate and modified aryloxyaminopropanol pharmacophore represent a suitable model of MTDL molecules, for which their β-adrenolytic and anticholinesterase effects have been confirmed.

Keywords: MTDL, synthesis, aryloxyaminopropanols, carbamates, pharmacological testing

SYNERGISTIC ANALGESIA: EXPLORING INTERACTIONS BETWEEN NON-OPIOID DRUGS AND CANNABINOID SYSTEM MODULATORS

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Background: In recent decades, growing interest has emerged around the potential synergistic use of cannabinoid receptor modulators with analgesics from other drug classes. Current evidence suggests that the endogenous cannabinoid system may contribute to the analgesic mechanisms of certain non-opioid agents. However, limited data exist on the potentially additive or synergistic effects that may result from co-administration of non-opioid analgesics with cannabinoid system modulators. The objective of this study was to evaluate the antihyperalgesic effects of cannabinoid system modulators—including a CB_1 receptor agonist and CB_1 receptor antagonist —in combination with acetaminophen or dipyrone in a rat model of inflammatory pain.

Material/Methods: Inflammation and hyperalgesia were induced by intraplantar injection of carrageenan into the hind paw. Thermal nociception was assessed using the hot-plate test, measuring paw withdrawal latency. Animals received dipyrone, acetaminophen, or the CB_1 receptor agonist WIN 55,212-2, alone or in combination with the CB_1 receptor antagonist AM-251, which was also tested independently.

Results: Dipyrone and WIN 55,212-2 each produced significant antinociceptive effects in the hot-plate test, whereas paracetamol did not elicit a significant effect on its own. The CB_1 receptor antagonist AM-251, which had no antinociceptive effect when administered alone, completely blocked the effect of WIN 55,212-2, and significantly attenuated the effects of both dipyrone and paracetamol, suggesting a modulatory role of CB_1 receptors in their analgesic mechanisms.

Conclusions: These findings support the involvement of the endocannabinoid system—particularly CB_1 receptors—in the analgesic effects of dipyrone and acetaminophen. The ability of AM-251 to attenuate their effects highlights the potential contribution of cannabinoid pathways to non-opioid analgesia, offering a basis for future research into combination therapies targeting the endocannabinoid system.

Keywords: cannabinoids,non-opioids, pain, efficacy, animals

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MUSCLE-SPECIFIC MICRORNAS AS DIAGNOSTIC AND THERAPEUTIC TARGETS FOR SARCOPENIA

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Background: Sarcopenia is a progressive skeletal muscle disorder characterised by the loss of muscle mass, strength, and function due to ageing. It increases the risk of falls, frailty, disability, and mortality in older adults. Management of sarcopenia requires a multidisciplinary approach beyond orthopedists and endocrinologists. It involves geriatricians, internists, rheumatologists, diabetologists, gynaecologists, cardiologists, physiotherapists, and nutrition specialists. In collaboration with the 5th Internal Medicine Clinic of LFUK and UNB, our Clinical Research Unit focused on identifying a sarcopenia-associated microRNA (miRNA) expression profile to improve diagnosis and quantify the degree of muscle performance decline.
Material/Methods: Eighty patients aged 55-86 years hospitalised at the 5th Internal Medicine Clinic were enrolled, all exhibiting varying degrees of reduced muscle performance. Participants were stratified according to their Short Physical Performance Battery (SPPB) scores. Based on our hypothesis that sarcopenia alters the expression of muscle-specific microRNAs (so-called myomiRNAs), we measured their levels in blood plasma. We analysed the expression of eight myomiRNAs, miRNA-29a, miRNA-29b, miRNA-1, miRNA-133a, miRNA-133b, miRNA-206, miRNA-208b, and miRNA-499 using the RT-qPCR method and correlated the results with clinical indicators of disease.

Results: We identified a distinct miRNA signature potentially serving as a biomarker for sarcopenia. Patients with low muscle performance showed increased expression of miRNA-1, miRNA-29a, and miRNA-29b levels and decreased expression of miRNA-206, miRNA-133a, miRNA-133b, miRNA-208b, and miRNA-499.

Conclusions: Our findings suggest that specific circulating miRNAs may be helpful in the diagnosis of sarcopenia and could serve as potential targets for gene-based therapies. Further **clinical studies** are needed to clarify the **molecular mechanisms** responsible for miRNA expression changes in the pathogenesis and progression of sarcopenia.

Keywords: sarcopenia, microRNAs, biomarker

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OPTIMIZATION OF LINEZOLID DOSING IN HEMATOONCOLOGICAL PATIENTS WITH SUSPECTED OR CONFIRMED GRAM-POSITIVE SEPSIS BASED ON A POPULATION PHARMACOKINETIC MODEL

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Background: Given the substantial pharmacokinetic variability of linezolid and the uniform dosage regimens to date, the objective of this study was to develop a population pharmacokinetic model for linezolid in hematooncological septic patients in order to propose an optimized dosing strategy for a more effective attainment of PK/PD targets.

Methods: Analysis of therapeutic drug monitoring data from hematooncological patients treated with linezolid for suspected or proven sepsis was conducted using nonlinear mixed-effects modeling. Based on the constructed population pharmacokinetic model, the theoretical distribution of pharmacokinetic profiles at varying dosing regimens was simulated using the Monte Carlo method to compare the PK/PD target achievement rate.

Results: Totally, 197 linezolid serum concentrations from 22 patients were included in the analysis. Patient age was identified as the covariate most predictive of linezolid pharmacokinetics. In a 59-year-old patient (the median age in our study population), the volume of distribution of linezolid was 64.2 L and clearance was 12.1 L/h. During the first four days of treatment, linezolid clearance was reduced by 33 %.

Conclusions: The likelihood of attaining target PK/PD parameters was enhanced following dose individualization according to patient age, administration of a loading dose, and administration of linezolid as a continuous infusion. For this purpose, a dosing nomogram was devised.

Keywords: **age, oxazolidinone, Monte Carlo simulation, therapeutic drug monitoring**

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GREEN SYNTHESIS OF SILVER NANOPARTICLES FROM LAVENDER AND THEIR ANTIBACTERIAL ACTIVITY AGAINST RESISTANT UDDER PATHOGENS

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Background: The global rise of multidrug-resistant microorganisms has prompted increased interest in nanotechnology-based strategies to combat antimicrobial resistance. Among these, silver nanoparticles (AgNPs) have shown promising antibacterial properties. This study explores a green synthesis approach using dry lavender leaves to produce silver nanoparticles and evaluates their antibacterial activity against common udder pathogens in dairy cows.

Material/Methods: Silver nanoparticles (Lav-AgNPs) were synthesized by mixing silver nitrate solution with reducing agents extracted from dry lavender leaves in five concentrations: 50 µg/l, 100 µg/l, 150 µg/l, and 200 µg/l. Pure silver nanoparticles (AgNPs) at identical concentrations served as a control group, along with penicillin (PEN) at 10 µg/ml. The antibacterial efficacy was tested against β-lactam-resistant *Staphylococcus aureus* and *Streptococcus uberis* isolated from cows with mastitis. Inhibitory activity was assessed using the disk diffusion method.

Results: Lav-AgNPs demonstrated a concentration-dependent inhibitory effect. At 150 µg/l and 200 µg/l, inhibition zones for *Staphylococcus aureus* ranged from 9 to 13 mm, while for *Streptococcus uberis*, zones ranged from 12 to 14 mm. In contrast, penicillin produced a consistent inhibition zone of only 5 mm for both pathogens.

Conclusions: The green synthesis of silver nanoparticles using lavender leaves offers an effective antibacterial alternative to conventional antibiotics. Lav-AgNPs exhibit significant inhibitory activity against antibiotic-resistant udder pathogens, highlighting their potential in applications for managing mastitis.

Keywords: silver nanoparticles, green synthesis, antimicrobial resistance, mastitis, lavender extract

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THERAPEUTIC DRUG MONITORING OF ANTIEPILEPTIC MEDICATIONS: A RETROSPECTIVE ANALYSIS OF LONG-TERM TREATMENT OUTCOMES

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Background: Epilepsy is a chronic disease affecting the brain, accompanied by episodes of involuntary movements that can vary in nature and frequency. Achieving therapeutic concentrations of antiepileptic drugs is a key aspect of effective treatment. Therefore, regular monitoring of drug concentrations is important to ensure an optimal therapeutic response. The aim of this study was to retrospectively evaluate the measured concentrations of one of the following antiepileptic drugs: valproic acid, carbamazepine and levetiracetam in patients on long-term treatment.

Material/Methods: Data were collected from the medical records of 282 patients treated with valproate, carbamazepine or levetiracetam in the years 2020-2022 at the University Hospital in Nitra. The data were statistically evaluated using IBM SPSS v.19.

Results: The dataset consisted of 282 patients with a mean age of 49.31 ± 1.04 years. The group of patients whose valproate levels were monitored consisted of 102 patients with a mean age of 45.74 ± 1.49 years. The patients were mainly treated for psychiatric illnesses (88.24%) and were hospitalized (80.40%). A subtherapeutic concentration was found in 40 patients (39.20%) and a concentration higher than the therapeutic range in 8 patients (7.80%). Carbamazepine levels were determined in 75 patients with a mean age of 50.35 ± 1.97 years, 53.30% of whom were treated as outpatients. Of these, 52% of the patients were treated for neurological disease. We found that 5 patients (6.70%) had lower, and 5 patients (6.70%) had higher drug concentration than the reference range. A total of 105 patients with a mean age of 52.08 ± 1.87 years were set up for levetiracetam treatment. Most of the patients (57.10%) were treated as outpatients, mainly for neurological disease (82.80%). Subtherapeutic drug concentration was observed in 28 patients (26.70%) and high concentration in 6 patients (5.90%).

Conclusion: When determining and adjusting the dosage of antiepileptics, it is important to consider the patient's demographic, anthropometric, and clinical data, including main diagnosis, associated diagnoses, and concomitant pharmacotherapy.

Keywords: antiepileptics, valproate, carbamazepine, levetiracetam, therapeutic drug monitoring

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