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Molecular Tumour Boards and Precision Medicine in Czech Oncology Centres

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Abstract The success and clinical response of patients to cancer treatment depends on the ability to identify somatic mutations associated with the tumour—the so-called mutation charge, according to which we can better target and individualise treatment. Next-generation sequencing (NGS) has become a revolutionary method for decoding the order of individual nucleotides and DNA fragments. Sequencing the human genome can be done much faster at present, the cost of sequencing is still falling, and insurance companies are also gradually adding this procedure to their reimbursement list. NGS has opened up completely new possibilities in therapeutic, diagnostic, and screening procedures at the level of individual patients, and not only in oncology. Groups of experts who consult on individual cases and the results of NGS with a certain regularity were formed to decide on personalised oncological care. Internationally, we can call them molecular tumour boards (MTBs), and the Czech Republic is not lagging in creating these groups. Large oncology centres and university hospitals approach precision medicine, and at the same time, they are aware of the need to share their data and knowledge. Therefore, the trend is toward the emergence of infrastructures and shared databases, which further validate, simplify, and accelerate the procedures. However, the lack of international communication, the failure of the NGS itself or proceeding with the NGS, and the subsequent reimbursement of the recommended treatment remain limiting factors for MTB. The presentation focuses on the activities of a particular MTB at the Masaryk Memorial Cancer Institute in Brno, which has been indicating patients for precision medicine since October 2019.

Keywords *oncology – precision medicine – molecular tumour boards*

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Safety and Systemic Exposure of Hydroxychloroquine in Healthy Volunteers After Inhalation Administration

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Abstract The anti-inflammatory and antiviral effects of hydroxychloroquine have not been demonstrated in clinical trials for the treatment of COVID-19. A possible reason for the failure is low local exposure in the lungs after p.o. or i.v. administration. In the Phase 1 study, the aim was to test the pharmacokinetics and safety of inhaled hydroxychloroquine after nebulization. In 6 healthy volunteers, we determined the pharmacokinetics after a single administration of 10 mg hydroxychloroquine from 19 blood samples collected at the following times: before nebulization and 2, 5, 10, 15, 20, 25, 30, 45, and 60 min, and 1:15, 1:30, 2:00, 2:30, 3:00, 4:00, 8:00, 12:00, and 24:00 hr after the start of nebulization. On Day 5, we determined the pharmacokinetics after repeated administration of 20 mg once daily from 12 samples taken at the following times: before nebulization and 5, 10, 20, 30, 45, and 60 min, and 2, 4, 8, 12, and 24 hr after the start of nebulization. Safety was assessed by observing biochemical parameters, blood count, and clinical parameters including ECG and blood pressure. After a single administration, the mean ± SD Cmax, T1/2, and AUC0-24 values were 5633.6 ± 2924.8 pg/ml, 11.7 ± 1.3 hr, and 6588.4 ± 1252.2 pg/ml*hr, respectively. After repeated administration on Day 5 the respective values were 4807.7 ± 1708.7 pg/ml, 14.83 ± 3.41 hr, and 26801.0 ± 8217.8 pg/ml*hr. We found no significant difference between the pretreatment and posttreatment values of safety parameters and they did not deviate outside the physiological range. We observed only minor adverse events related to the bitter taste of hydroxychloroquine. There were no systemic or respiratory adverse reactions. We have demonstrated acceptable safety of the inhaled route of administration of hydroxychloroquine in this dose group.

Keywords *COVID-19 – drug development – nebulization – pharmacokinetics*

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The Effect of Dapagliflozin, Pioglitazone, and Their Combination in the Development of Inflammation and Fibrosis in the Pathogenesis of Experimental Diabetic Nephropathy

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Abstract Diabetic nephropathy (DN) is a leading cause of end-stage renal failure. Chronic inflammatory processes and subsequent renal tissue fibrotization result in destruction of normal kidney structure and functional deterioration. Dapagliflozin (Dapa) and pioglitazone (Pio) have been shown to prevent progression of diabetes complications in several organ systems in both Type 1 (T1DM) and Type 2 diabetes mellitus. We investigated whether the simultaneous PPAR–γ activation and SGLT2 inhibition alleviate significantly fibrotic and inflammatory processes and thus slow DN progression in early stages of T1DM. Experimental diabetes was induced in rats by streptozotocin (STZ, 55 mg/kg, i.p.). STZ animals were treated with either Dapa (10 mg/kg), Pio (12 mg/kg), or their combination for 6 weeks. Drugs were mixed in rat chow. Controls and the STZ group received standard chow. Excised kidneys were used for histological and immunohistochemical staining and for the assessment of gene and protein expression of inflammatory and profibrotic factors. Hyperglycaemic conditions led to the elevated gene and protein expression of pro-inflammatory markers (COX2, TNFα, IL1b, IL6) in untreated STZ rats compared to the controls. We also noted upregulated expression of profibrotic Col1a1. Kidney sections of STZ rats examined by immunohistochemical analysis showed an increase in α-SMA immunopositive areas. Histological examination revealed development of glomerulosclerosis associated with interstitial fibrosis and hypertrophy of glomeruli. Dapa and Pio monotherapy markedly reduced expression of all inflammatory markers compared to the STZ rats. We observed a downregulation in Col1a1 expression, a decline in α-SMA positive areas, and restoration of histopathological changes. The effect of combination treatment indicated a declining trend in inflammatory processes. Renal damage within histological examination was significantly attenuated compared to the STZ group, reaching only an effect of monotherapies. This study suggests that Dapa and Pio have an evident renoprotective character when used in monotherapy, but do not exhibit the expected synergistic effect in modulating inflammatory and fibrotic mechanisms.

Keywords *inflammation – fibrosis – glomerulosclerosis – Type 1 diabetes mellitus – rat*

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Genotypic Resistance of *Mycobacterium Tuberculosis* to First- and Second-line Antituberculosis Drugs: What Is the Situation in Slovakia and the Czech Republic?

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Abstract Tuberculosis (TB), a disease caused by *Mycobacterium* (*M.*) *tuberculosis*, represents one of the most dangerous infections worldwide. The incidence of drug-resistant forms of TB is increasing year by year; therefore, developing new methods for rapid diagnostics is necessary. The future of diagnosis of resistant forms of TB is represented by whole genome sequencing (WGS), which allows us to characterise all gene variants associated with resistance to all antituberculotics at a clinically relevant time. We performed WGS on 81 *M. tuberculosis* samples collected between 2017 and 2020 in Slovakia and the Czech Republic to characterise the most frequent gene mutations encoding resistance to first- and second-line antituberculotics. Variant calling analysis classified 31 (38.3%) strains as sensitive to all antituberculotics, 3 (3.7%) strains monoresistant to isoniazid, 1 (1.23%) strain polyresistant to isoniazid and streptomycin, 45 (55.6%) strains as multidrug resistant, and 1 (1.23%) strain as extensively drug resistant. The most frequent mutations encoding resistance were *katG* S315T for isoniazid, *rpoB* S450L for rifampicin, *embB* M306I for etambutol, *pncA* L182S for pyrazinamid, *rpsL* L88A for streptomycin, *gyrA* A94G for fluoroquinolones, *rrs* 1401a> g for second-line injectable aminoglycosides, and *ddn* Trp-88-STOP for delamanid. The overall sensitivities and specificities for WGS were 100% and 100% for rifampicin, 100% and 100% for isoniazid, 98.47% and 25% for ethambutol, 97% and 76% for pyrazinamide, 97% and 83% for streptomycin, 79% and 81.2% for second-line injectable aminoglycosides, and 95.85% and 94% for fluoroquinolones. This study offers a comparison of the determination of *M. tuberculosis* resistance by WGS with phenotypic DST in Slovakia and the Czech Republic. The high concordance between these methods highlights the utility of WGS as a high-resolution approach in the diagnosis and characterisation of resistance patterns of drug-resistant TB.

Keywords *tuberculosis – resistance – MDR – XDR – whole genome sequencing*

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Analysis of the Role of Necroptosis and Its Interconnection With Autophagy in Acute Myocardial Ischemia/reperfusion Injury

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Abstract Necroptosis, regulated via receptor-interacting protein kinase 3 (RIP3) and mixed lineage domain kinase domain-like pseudokinase (MLKL), plays a prominent role in mediating myocardial ischemia/reperfusion (IR) injury. However, the extent to which necroptosis contributes to such damage under short reperfusion remains elusive. Thus, we aimed to evaluate whether necroptosis mediates acute myocardial IR and simultaneously to identify its linkage with autophagy. Langendorff-perfused rat hearts subjected to 30-min ischemia followed by 10-min reperfusion exhibited impaired cardiac function that was not ameliorated by RIP3 inhibition. Immunoblotting analysis revealed that the detrimental effects of IR were unlikely mediated by necroptotic cell death, as neither the canonical RIP3–MLKL pathway nor the proposed noncanonical molecular axes involving CaMKII –mPTP (calcium/calmodulin dependent protein kinase II –mitochondrial permeability transition pore) and PGAM5–Drp1 (phosphoglycerate mutase 5–dynamin-related protein 1) were activated. Although the signalling involved in autophagy inhibition was not affected, autophagy activation was suppressed by IR as evidenced by decreased protein expression of Beclin-1, pSer555-ULK1, pSer555-ULK1/ULK1 ratio, and LC3-II/LC3-I ratio. RIP3 inhibition prevented the plasma membrane rupture and delayed mPTP opening, which was associated with modulation of xanthin oxidase (XO) and manganese superoxide dismutase (MnSOD). Additionally, LC3-II expression in IR hearts was suppressed by RIP3 inhibition, indicating some effect on autophagosome processing, but this pharmacologic intervention significantly altered no other signalling involved in either autophagy activation or inhibition. In conclusion, this is the first study suggesting that RIP3 regulates early reperfusion injury via oxidative stress and mitochondrial activity-related effects, rather than necroptotic cell death. In addition, we also showed that the relationship between this pro-necroptotic kinase and autophagy under such acute IR settings is unlikely, apart from the potential impact on autophagosome regulation.

Keywords *necroptosis – autophagy – ischemia/reperfusion injury – oxidative stress*

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Vancomycin Pharmacokinetic in Patients Treated With Intermittent Hemodialysis

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Abstract Even though vancomycin is frequently used in dialysis patients, dosing strategy and therapeutic drug monitoring (TDM) targets are still poorly standardised in this patient group. Patients might be initially underdosed due to administration of insufficient loading dose (LD) and subsequently overdosed due to administration of large maintenance doses when low plasmatic levels are measured in the beginning of the treatment. We present PK analysis based on the TDM data obtained from daily routine practise in two dialysis centres. Data from 118 subsequent patient cases are included. Based on this analysis we formulate dosing recommendations to obtain effective therapy from the beginning of vancomycin administration.

Vancomycin volume of distribution was 0.87 L/kg, interdialysis elimination constant was 0.0073 h^{-1}, and average half-life between dialysis sessions was 95 hr. In patients treated by dialysis for less than 3 months, high variability of PK parameters was observed. If we excluded this group of patients from the analysis, we observed slightly positive linear correlation of vancomycin clearance and length of dialysis treatment—probably an adaptation of the organism to end-stage renal failure. If the haemodialysis (HD) session was performed after the distribution phase, there was a relatively small difference in amount of vancomycin eliminated during the HD session when high- and low-flux membranes were used (20.88% and 12.86%, respectively). In contrast, if vancomycin infusion was administered at the end of the HD session (still frequent practise in some HD centres), a large and unpredictable amount of the drug was eliminated. The (re)distribution phase after the HD session or after vancomycin administration takes 5 hr. Sufficient drug exposition (AUC 400 mg*h/L) is achieved by targeting plasmatic levels of 15 to 25 mg/L before HD sessions. To obtain these levels on the second day of the therapy, we calculated that LD should be 1,500 mg for patients weighing < 100 kg and 2,000 mg for patients weighing > 100 kg. In our data set, patients with lower body weight were underrepresented so our approach might not be valid for patients weighing less than 60 kg. We strongly recommend not administering vancomycin (and other dialysable drugs) during the HD session.

Keywords *therapeutic drug monitoring – loading dose – end-stage kidney disease – redistribution*

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70th Czech-Slovak Pharmacological Days

Benzofuranylpropylaminopentane During Chronic Mild Stress in Rats

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Abstract Selegiline, which is used in clinical practise as an antiparkinsonian drug, has a wide range of other effects. Together with benzofuranylpropylaminopentane (BPAP), they act as enhancers of catecholaminergic and serotonergic neurotransmission in very small doses, which could have a positive effect on neuronal survival. In addition to these neurotransmitter systems, enhancers also affect glutamatergic neurotransmission, which plays a key role in brain plasticity. Neuronal integrity can be disrupted by various factors, such as the excessive stress that we have to cope with during our lives. In preclinical studies, the various animal models are used to model stressful situations, including the model of chronic mild stress, which adequately reflects everyday situations in human life. The aim of the study is to test the hypothesis that selegiline and BPAP induce changes in parameters related to brain plasticity during chronic stress. In the present ongoing study, we are using the 4-week-long protocol of chronic mild stress that includes several different stress stimuli to which the animals are exposed each day of the experiment. Simultaneously, the animals are subcutaneously administered selegiline and BPAP in enhancer-specific and enhancer-nonspecific doses for 3 weeks. At the end of the experiments, the rats will have their blood serum, prefrontal cortex, and hippocampus collected to analyse endocrine parameters and gene expression of selected genes related to brain plasticity.

Keywords *enhancer drug – chronic mild stress – brain plasticity*

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Therapeutic Strategy in Patients With Familiar Form of Hypertrophic Cardiomyopathy: a Pilot Study

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Abstract Hypertrophic cardiomyopathy is an autosomal dominant inherited disease of the myocardium caused by sarcomeric mutations. Sudden cardiac death can be the most dreaded manifestation of the disease. The most common mutations were identified in myosin heavy chain (MHC), myosin binding protein C (MYBPC3), and cardiac troponin T (cTnT). Aetiology of the disease is also connected with undiscovered genes and other environmental factors. The prognostic value of mutations is proportional to the degree of myocardial hypertrophy penetrance. With improvement in genetic analyses, the pharmacological therapy of genotype positive–phenotype negative patients is a future goal to slow phenotype expression.

We prospectively examined 40 patients with genetically confirmed diagnosis of hypertrophic cardiomyopathy. All patients underwent noninvasive cardiac evaluation. DNA was extracted from the whole blood samples. Exon 9 from the locus TNNT2 was sequenced. The heterozygous genotype 318CtoT was found in 17 patients and the homozygous genotype 318CtoT was found in 17 patients as well. We detected no mutation in 6 patients. The aim of the study was to compare the therapeutic strategy in homozygous and heterozygous mutation carriers.

Pharmacological therapy, mostly beta blockers and calcium channel blockers, was used in 64.7% (11/17) of homozygous as well as heterozygous mutation carriers, whereas 23.5% (4/17) of TNNT2 homozygotes were implanted with a cardioverter-defibrillator in the primary or secondary prevention of sudden cardiac death in comparison with only one of the heterozygous patients. The subgroup of homozygotes also demonstrated a higher incidence of syncopal episodes. The myocardial septal ablation was more frequent in heterozygotes.

The study results indicate a worse prognosis in TNNT2 homozygotes for the higher risk of sudden cardiac death in comparison with heterozygous mutation carriers. Disorganisation of the myocardial architecture seems to be the pathological substrate of life-threatening arrhythmias. Further studies are necessary to evaluate the prognostic value of genotype–phenotype correlation in TNNT2 mutation carriers.

Keywords *hypertrophic cardiomyopathy – sarcomeric mutations – therapy*

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Suppression of Anthracycline Cardiotoxicity by Dexrazoxane Is Not Mediated by Its Metal Chelating Metabolites in Rabbits and Rat Cardiomyocytes

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Abstract Anthracycline cardiotoxicity has traditionally been attributed to iron-catalysed direct oxidative injury. Dexrazoxane, the only cardio protectant approved for this indication, was thought to prevent cardiotoxicity via its metal-chelating metabolite ADR-925. However, this hypothesis lacks direct supporting evidence and was recently challenged by the topoisomerase IIβ (TOP2B) hypothesis. This study thoroughly examined the putative role of iron-chelating metabolites in dexrazoxane cardio protection and investigated alternative TOP2B-related mechanisms. We performed a pharmacokinetically guided study on the protective effects of dexrazoxane and exogenously administered ADR-925 against daunorubicin-induced cardiotoxicity in vitro in neonatal ventricular cardiomyocytes (NVCM) and in vivo in a chronic rabbit model. Next, we compared the effects of dexrazoxane and ADR-925 on TOP2B and assessed daunorubicin-induced DNA damage. The intracellular concentrations of ADR-925 in NVCMs and rabbit hearts after treatment with exogenous ADR-925 were similar to or greater than those observed after treatment with the parent compound dexrazoxane. However, treatment with ADR-925 and intermediate metabolites of DEX provided no significant cardio protection against anthracycline cardiotoxicity, whereas dexrazoxane exhibited high cardioprotective efficiency. Unlike dexrazoxane, ADR-925 did not prevent daunorubicin-induced mortality, heart failure, an increase in cardiac troponin T levels in plasma, or myocardial histopathology. Dexrazoxane, but not ADR-925, inhibited and depleted TOP2B and prevented daunorubicin-induced genotoxic damage. TOP2B dependency of the cardioprotective effects was probed and further supported with diastereomers of a close DEX derivative. This study strongly supports a new mechanistic paradigm that attributes clinically relevant cardio protection against anthracycline cardiotoxicity to interactions with TOP2B, but not metal chelation and protection against direct oxidative damage.

Keywords *anthracyclines – cardio protection – dexrazoxane – metal chelation – topoisomerase IIβ*

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Immune-mediated Adverse Effects of Checkpoint Inhibitors: a Clinical Experience

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Abstract Systemic antitumour immunotherapy with monoclonal antibodies (immune checkpoint inhibitors [ICI]) was one of the biggest breakthroughs in the pharmacotherapy of solid tumours in the past decades. Unfortunately, their use might result in rare but life-threatening autoimmune adverse effects. Expansion of some T-lymphocytes subsets is probably involved in the immune-related adverse effect (irAE) development, but detailed mechanisms of these irAEs have been investigated insufficiently so far. We performed a retrospective analysis of irAEs of the ICIs used at the Masaryk Memorial Cancer Institute, a tertiary comprehensive oncology centre, in the years from 2011 to 2021. The ICIs were administered in 648 patients, with the most frequent indications being malignant melanoma, renal carcinoma, and lung carcinoma (235, 144, and 114 cases, respectively). The most commonly used ICI was nivolumab (316 patients), followed by pembrolizumab and ipilimumab (93 and 68 patients, respectively). irAEs with the necessity of immunosuppressive treatment were pronounced in 83 patients (12.8%). Further, we report a case of serious irAE after treatment with a combination of ipilimumab and nivolumab for renal clear cell carcinoma. Autoimmune thyroiditis Grade 2 and colitis Grade 4 developed. Thyroiditis resulted in hypothyreosis, which was treated with supplementation with levothyroxine. Prednisone, mesalazine, octreotide, infliximab, vedolizumab, and mycophenolate mofetil were used to manage G4 colitis, with partial response. The further treatment was symptomatic. The occurrence of irAEs in the patients treated at Masaryk Memorial Cancer Institute was comparable with the literature data. Early diagnosis and appropriate management of irAE are essential for further oncological treatment.

Keywords *immune checkpoint inhibitors – immunotherapy – adverse effect – autoimmunity*

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The Effect of Salidroside on Liver Cytochrome P450

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Abstract Salidroside is the main active ingredient of *Rhodiola rosea* (RR), an herb with clinically documented antidepressant, antistress, anxiolytic, and antifatigue central effects. A variety of over-the-counter herbal products containing RR can be purchased in Czechia. Cytochrome P450 (CYP) is an enzyme with an essential role in the metabolism of many drugs. Various CYP–herb interactions were described, and some of them are potentially dangerous for patients. The effect of RR or salidroside on CYP remains unclear: Our project aimed to evaluate it.

Wistar albino rats (n = 40) were administered with salidroside intragastrically at doses of 5, 15, and 45 mg/kg/day or with a vehicle (water) for 7 consecutive days. Liver samples were collected 24 hr after the last dose. Liver microsomes were isolated by differential ultracentrifugation. To evaluate the metabolic activity (MA) of liver microsomes, the incubations with CYP-specific substrates (diclofenac—CYP2C6, testosterone—CYP2A, CYP3A, CYP2C, CYP2B, phenacetine—CYP1A2, and dextromethorphan—CYP2D1/2) were performed. Based on the alterations of MA observed, the quantity of CYP2C6 and CYP1A2 proteins in the liver microsomes was evaluated by western blot. To assess the effect of salidroside on nuclear receptor PXR, HepG2 cells were transfected with a luciferase reporter construct and either rat or human expression vector for PXR.

After the subchronic administration of a dose of 5 mg/kg/day, salidroside increased the MA of CYP2C6 and CYP1A2. Western blot analysis did not reveal a significant change in the amount of protein except the reduction in CYP2C6 after the administration of 45 mg/kg/day. Salidroside inhibited the activation of rat and human PXR in luciferase reporter assays. Our results indicate that salidroside, the main active ingredient of the RR, has a low interaction potential with CYP. However, further research is needed to confirm the safety of salidroside in clinical practise.

Keywords *cytochrome P450 – liver microsomes – Rhodiola rosea – salidroside*

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Pro-apoptotic Potential of *Pseudevernia Furfuracea* Lichen Extract and Their Metabolite Physodic Acid

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Abstract The pharmaceutical potential of lichens and their secondary metabolites has recently attracted increasing attention worldwide. Although chemotherapy is a common treatment for cancer, it has a wide range of serious side effects, including myelo- and immunosuppression, hepatotoxicity, and neurotoxicity. Combination therapies using natural substances are widely recommended to attenuate the adverse effects of chemotherapy. The aim of this study was to investigate the antileukemic potential of extract from the lichen *Pseudevernia furfuracea* (L.) Zopf (PSE) and isolated physodic acid (Phy) in an in vitro acute lymphoblastic leukemia (ALL) model. Our results showed that PSE and Phy treatment induced apoptosis in Jurkat cells in a dose- and time-dependent manner. We confirmed that ROS production and consequent DNA damage played an important role in PSE- and Phy-mediated apoptosis. Moreover, the DNA repair mechanism, including the phosphorylation of ATM, H2A.X, and SMC1 proteins, was subsequently activated, followed by p21, p53, and p27 activation and cell cycle arrest. Moreover, PSE and Phy treatment led to the phosphorylation of MAPK signalling, including p38 MAPK, JNK, and PI3K/Akt. Furthermore, minimal or no cytotoxicity in normal peripheral lymphocytes supports the use of PSE and Phy as antileukemic agents. However, future studies might be needed to determine more associated mechanisms of the anticancer action of the tested substances more deeply.

Keywords *Pseudevernia furfuracea – physodic acids – apoptosis – oxidative stress – MAPK*

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The Potential of Targeted Metabolomics in Biomedical Research

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Abstract The metabolomics presents a comprehensive analysis of low molecular weight metabolites (< 1500 Da), which play a crucial role in biological systems as signalling molecules, energy sources, and metabolic intermediates. We can discover and validate metabolomic indicators of many diseases and their animal models using current metabolomics techniques. Various complementary analytical methodologies such as mass spectrometry coupled with liquid or gas chromatography and nuclear magnetic resonance (NMR) are employed to identify and quantify as many metabolites as possible.
This study presents several mass-spectrometry-based targeted metabolomics analyses performed in our laboratory. Twenty-two plasma metabolites were significantly changed in ovalbumin-sensitised guinea pigs (an animal model of allergic asthma) and nonsensitised naïve guinea pigs, including phosphatidylcholines, carnitine, symmetric dimethylarginine, tryptophan, and taurine. Metabolomic characterisation of melanoma-bearing Libechov minipig (an animal model for melanoma research with progressing and spontaneously regressing melanomas) revealed a significantly altered metabolomic profile. Forty-two metabolites were differentially regulated in plasma samples, pointing to alteration of arginine, glycerophospholipid, and acylcarnitines metabolism. Finally, using targeted metabolomics analysis, we were able to differentiate 2D and 3D cell cultures of the novel pancreatic cancer cell line isolated from a patient with pancreatic ductal adenocarcinoma (PANDA). Metabolomics, parallel to genomics or proteomics, has the potential to discover biomarkers and help us understand a variation in molecular mechanisms, which highlights its application in biomedical research and precision medicine.

Keywords *metabolomics – biomarkers – mass spectrometry*

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Parenteral Depot Dosage Forms in Psychiatry and the Development of a New Particulate Dosage Form of Mirtazapine

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Abstract Depot dosage forms are gaining importance in psychopharmacology and are more effective than conventional p.o. dosage forms. Depot dosage forms are associated with a lower risk of relapse in psychotic illnesses but have similar potential in other neuropsychiatric illnesses. Depot dosage forms can provide balanced plasma levels and might prevent toxicity plasma concentrations that are too high or, conversely, ineffectiveness at plasma levels that are too low. The main advantages of depot psychopharmaceuticals include improved adherence to treatment, no problems with absorption from the gastrointestinal tract, bypassing first-pass metabolism, and no risk of accidental or intentional overdose. The development of depot dosage forms is currently focused on the study of microparticles.

Based on preformulation experiments, suitable formulation and process parameters were selected for the preparation of microparticles based on lactic acid-glycolic acid copolymer (PLGA) for parenteral administration. The prepared microparticles were evaluated for drug content, morphology, and dissolution test. Six different drug release profiles were prepared by suitable procedures and subsequently two profiles were selected for administration to laboratory animals (rats).

On the basis of the dissociation behaviour of the prepared microparticles under in vitro conditions, a novel drug formulation containing mirtazapine was administered i.p. to the laboratory rat with the assumption of administration once a week. Based on frequent sampling, the pharmacokinetic profile of the administered microparticles was determined using a previously developed and validated HPLC method. The serum was further used to determine liver function tests, lipid spectrum, and selected adipokines.

A depot dosage form of mirtazapine with an established pharmacokinetic profile was obtained. According to biochemical analysis, we observed minimal effect on ALT and AST levels. In the medicated group, we observed slight differences from the control group in the lipid spectrum and in the levels of selected adipokines.

Keywords *depot dosage forms – mirtazapine – neuropsychiatric illness – nonadherence*

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Intra- and Intersubject Variability in Preclinical Pharmacokinetic Studies: Advantage of Crossover Vs. Parallel Design in Short-term Comparative Pharmacokinetic Studies in Rats

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Abstract Due to many factors affecting in vivo experiments, researchers have to deal with the impact of variability (both intra- and interindividual) on the accuracy of results. Traditionally, parallel design is usually adopted for short-term preclinical pharmacokinetic studies. In our analysis, we focused on whether it is possible to mitigate the effects of variability by employing crossover design rather than parallel design. We examined the data from previously performed experiments with a formulation of abiraterone acetate, which was orally administered to six groups of rats (A–F) in a parallel setting. Another group of rats was dosed with the same formulation repeatedly (in two following periods) with the aim to assess the intraindividual variability (IIV). There was no statistical difference between the geometric mean (90% confidence interval) of abiraterone AUC_{last} of groups A–F and the IIV group (24.36 [23.79, 41.00] vs. 26.29 [20.56, 47.00] mg/mL.min.g). In spite of this apparent similarity, the values of abiraterone AUC_{last} of isolated parallel groups were moving randomly over a wide range from 9.62 to 44.62 mg/mL.min.g. Moreover, a value of 100% was not included in confidence intervals for 4 out of 15 pair comparisons between the groups in a parallel setting. Therefore, in those cases the true ratio would be falsely rejected. Simulations where CV obtained from our experiments was used showed that for a crossover design, there is much higher probability that the resulting ratio would lie in the range from 80% to 125%, which is the standard bioequivalence acceptance range (76% probability vs. 49% for crossover vs. parallel design, respectively). Based on these findings, we conclude that a crossover design could assure more accurate and precise results in contrast to parallel design in short-term comparative preclinical pharmacokinetic studies.

Keywords *variability – pharmacokinetics – crossover design*

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Risk Perception of Treatment and Prevention of Covid-19 Disease in Slovakia

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Abstract The COVID-19 pandemic has resulted in more than 6.2 million deaths worldwide. Despite advances in the treatment of this disease over the last 2 years, the only way out of the pandemic is vaccination against the SARS-CoV-2 virus, which is constantly confronted with considerable mistrust and fears of adverse reactions (ADRs) in Slovakia. The aim of this study is to analyse reported suspicions of ADRs of registered COVID-19 vaccines (Comirnaty, Vaxzevria, Spikevax), which the State Institute for Drug Control received from health care professionals and patients between 1 January and 31 May 2021. The program R (version 3.6.3), a language and environment for statistical calculations, GNU GPL license was used for data analysis.

During the evaluation period, 5,763 reported suspicions of ADRs were analysed. Overall, there was a significant ($p < .0001$) fivefold increase in the number of reported ADRs. Ninety-three percent of ADRs ($n = 5,346$) were reported for COVID-19 vaccines. No statistically significant difference ($p \leq .238$) was identified between Spikevax and Comirnaty in the proportion of serious ADRs. However, a significantly higher ($p \leq .00001$) proportion of reported suspicions of severe ADRs was observed with Vaxzevria. There is a significant difference in the ratio of serious ADRs between the sexes for all COVID-19 vaccines ($p < .00001$); in women this ratio is in all cases significantly higher than in men ($p < .0001$). ADRs were most often reported by patients ($p < .0001$).

In Slovakia, the rate of spontaneous reporting of suspected ADRs has been low for a long time; in the period between January and May 2021, however, the rate increased as a result of active calls for ADR reporting, most often from patients. According to European data, Vaxzevria has a significantly higher ratio of reported suspicions of serious ADRs. For all vaccines, the incidence of severe ADRs is significantly higher in women.

Keywords *COVID vaccines – Spikevax – Comirnaty – Vaxzevria – spontaneous reports of adverse reactions*

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ACE2 Inhibitor MLN-4760 Enhances Noradrenalin-induced Contraction and Stimulates Endothelium-dependent Contraction of Femoral Arteries: the Benefit of Taxifolin Treatment

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Abstract The importance of disclosing the various effects of the inhibition of angiotensin-converting enzyme-2 (ACE2) in regulation of vascular tone has been shown during the COVID-19 pandemic. Various substances with antioxidant properties were proposed to have a beneficial effect in mitigating the progress of COVID-19. Therefore, we have investigated the effect of 14-day ACE2 inhibitor MLN-4760 treatment (s.c.) and 10-day oral coadministration of taxifolin (TAX) on the regulation of vascular tone of isolated femoral arteries. Our results showed that the ACE2 inhibitor MLN-4760 enhanced the maximal noradrenalin-induced contraction as well as the sensitivity to noradrenalin (NA). The acetylcholine-induced endothelium-dependent relaxation was not changed after MLN-4760 administration, but the acetylcholine-induced endothelium-dependent contraction was augmented. TAX administration reduced the maximal NA-induced contraction, but did not change the sensitivity to NA. The endothelium-dependent relaxation was improved after TAX application as shown by the calculation of area under the curve. TAX did not influence the acetylcholine-induced endothelium-dependent contraction. In conclusion, administration of MLN-4760 leads to increased NA-induced contraction of femoral arteries, which can be partially mediated through increased endothelium-dependent contraction. TAX mitigated the enhanced NA-induced contraction caused by MLN-4760 administration, but did not affect endothelium-dependent contraction.

Keywords *MLN-4760 – femoral artery – taxifolin – endothelium-dependent contraction –angiotensin-converting enzyme-2*

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Antiproliferative Effect of New Chalcone Derivative in Human Breast Cancer Cells: an In Vitro Study

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Abstract Despite significant advances in treatment, breast cancer (BC) mortality has not decreased significantly in recent decades. For this reason, it is necessary to find new selective antitumour drugs without cytotoxic effects on healthy tissues. Chalcones, synthesised in plants as precursors of flavonoids, are natural compounds with many biological effects, including antiproliferative and antitumour activity. Our study aims to elucidate the antiproliferative effect of synthetic chalcone ZK-CH-11d on human BC cells. Chalcone was found to be able to significantly inhibit tumour cell proliferation and cause cell death without affecting the nontumour line. This effect was associated with cell cycle arrest at the G2/M phase. Cell cycle arrest was accompanied by tubulin dysregulation and changes in regulatory protein levels such as cyclins, cyclin-dependent kinases, and important tumour suppressors. Chalcone-induced DNA damage also triggered cascades leading to the initiation of the intrinsic apoptosis pathway. In addition, chalcone caused inhibition of the PI3K/Akt/mTOR signalling pathway and increased AMPK phosphorylation, indicating initiation of autophagy. Progression of the chalcone-induced autophagy process was detected by analysis of proteins such as PTEN, Beclin-1, ULK1, and LC3A/B. Autophagy can be considered a prosurvival process but also a type of cell death. Chloroquine, an autophagy inhibitor, significantly enhanced the cytotoxic effect of ZK-CH-11d in MDA-MB-231 cells, and was used to verify the participation of autophagy in chalcone-induced cell death. It has been found that autophagy has been activated in cells as a defence mechanism and is not involved in chalcone-induced cell death.

Keywords *breast cancer – chalcone – antiproliferative – cell cycle – apoptosis – autophagy*

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Modulatory Effect of *Aesculus Hippocastanum* L. Extract on Fibroblasts Actively Participating in Tumour Progression and Wound Healing

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Abstract Fibroblasts play a key role in the formation of granulation tissue, tumour stroma, or both. These cells are able to differentiate into myofibroblasts, acquiring a highly contractile phenotype. Transforming growth factor β1 (TGF-β1) is considered to be the main inducer of fibroblast-to-myofibroblast differentiation. Previous studies have demonstrated the ability of *Aesculus hippocastanum* L. extract (horse chestnut extract [HCE]) to induce contraction forces in fibroblasts, a process with remarkable significance in tissue repair, and to exert antiproliferative and antiangiogenic properties. The published evidence motivated us to evaluate the effect of HCE on canonical (SMAD) and noncanonical (non-SMAD) TGF-β signalling in normal fibroblasts isolated from human healthy skin (human dermal fibroblasts [HDFs]) and in their malignant counterparts (cancer-associated fibroblasts [CAFs]) isolated from basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) using western blot and immunofluorescence (in vitro study). The in vivo study was performed on Sprague-Dawley rats with two inflicted wounds on their backs to evaluate the potential of HCE on wound healing. Skin incisions were processed for wound tensile strength (TS) measurement, and excisions were subjected to histological examination. On the in vitro level, HCE induced fibronectin deposition in BCCFs, an effect not seen in SCCFs. Furthermore, the presence of TGF-β1 led to activation of canonical signalling in HDFs and BCCFs, whereas it triggered noncanonical (AKT, ERK1/2) signalling in SCCFs. The animal study revealed that HCE increased wound TS and improved collagen organisation. In summary, specific differences observed in signalling of HDFs and CAFs should be considered in the development of new therapeutic strategies targeting wound and tumour microenvironments. Our study showed that HCE might be useful to improve healing of acute wounds. However, the use of an experimental rat model warrants a direct extrapolation to the clinical situation.

Keywords *horse chestnut – fibroblasts – tumour microenvironment – wound healing – TGF-β1*

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Tetrahydropyrolles as Potential Antitumour Compounds

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Abstract Nowadays, one of the main causes of mortality is cancer, and for that reason there has been increased interest in cancer treatment research. Chemotherapy is one of the therapeutic options, involving the use of natural and synthetic substances with the ability to block fast-growing tumour cells. The high toxicity and poor tolerance of current anticancer drugs emphasise the need to find new compounds with strong antiproliferative activity, low toxicity, and minimal side effects. Recent studies suggest that tetrahydropyrroles appear to be molecules with antitumour activity. Tetrahydropyrroles, also called pyrrolidines, are organic compounds found in many natural substances. Derivatives of pyrrolidines have shown antibacterial, neurological, and antiproliferative properties. They are able to affect tumour cells by several mechanisms, making them potential antiproliferative agents. The aim of this study was to investigate the effect of tetrahydropyrrole SS13 on colorectal cancer tumour cell lines (HCT116 and Caco-2). Metabolic MTT assay and BrdU proliferation assay were performed to verify the antiproliferative activity and determine the inhibitory concentration IC_{50}. In our experiments, the tested compound inhibited the tumour cells' growth with an IC_{50} of 7 μmol/L for HCT116 and 2.5 μmol/L for Caco-2. Pyrrolidine SS13-induced apoptosis was associated with caspase activation, PARP cleavage, and reduced mitochondrial potential accompanied by increased expression of the proapoptotic protein Bad and release of cytochrome c. Our findings indicate initiation of an intrinsic apoptosis pathway. Furthermore, SS13 induces DNA damage in the HCT116 and Caco-2 cells, which is probably the result of increased oxidative stress mediated by oxygen and nitrogen radicals. In conclusion, SS13 inhibits proliferation and induces apoptosis in both tumour cell lines, suggesting that it could represent a potential advance in cancer prevention and treatment.

Keywords *tetrahydropyrroles – antiproliferative effect – apoptosis*

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New Ligands of Nuclear Receptors as a Tool for the Study of Drug Metabolism Regulation

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Abstract The constitutive androstane receptor (CAR) and Pregnane X receptor (PXR) are known as the established nuclear receptors that regulate the expression of several key cytochrome P450 enzymes, predominantly CYP3A4 and CYP2B6. Recently it has been shown that the receptors have also essential roles in the regulation of endogenous metabolism of glucose, lipids, cholesterol, and bile acids. Unfortunately, currently known ligands of human or mouse CAR or PXR, either agonists or antagonists, are either poorly selective or indirect. The aim of this work is to present new ligands of mouse and human CAR and PXR that would enable a more detailed study of the receptor in advanced models.

Keywords *constitutive androstane receptor (CAR) – Pregnane X receptor (PXR)*

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Determination of Caffeine and Its Metabolites in Human Plasma Using Mass Spectrometry

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Abstract Caffeine belongs to the group of widely consumed psychostimulants with several mechanisms of action and various positive and negative effects on organisms. Caffeine undergoes extensive hepatic metabolism by partial demethylation and hydroxylation mediated primarily via the cytochrome P450 1A2 (CYP1A2) to form primary metabolites, namely paraxanthine (1,7-dimethylxanthine), theophylline (1,3-dimethylxanthine), theobromine (3,7-dimethylxanthine), and 1,3,7-trimethyluric acid. It is valuable to investigate caffeine's pharmacokinetics and pharmacodynamic properties to understand caffeine's effects better. The scope of this work was to develop and validate a sensitive and high-throughput analytical method for the simultaneous quantification of caffeine and its primary metabolites in plasma samples. An ultra-high-performance liquid chromatography coupled to a triple quadrupole mass spectrometer with multiple reactions monitoring method was developed. The analytes were extracted using a micro solid-phase extraction plate and separated over 4 min. The developed method was successfully validated according to the European Medicine Agency (EMA) guideline over a concentration range of 5 to 1,500 ng/mL for caffeine, 5 to 1,200 ng/mL for theobromine, and 2.5 to 1,200 ng/mL for theophylline, paraxanthine, and 1,3,7-trimethyluric acid. The developed and fully validated analytical method was applied to quantify caffeine and its four primary metabolites in human plasma after ingestion of caffeine capsules.

Keywords *caffeine – metabolism – methylxanthines*

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Therapeutic Drug Monitoring of the First-line Antituberculotics Using LC-MS/MS

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Abstract Until the global SARS-CoV-2 virus outbreak in 2020, tuberculosis (TB) was the leading cause of death from a single infectious agent. Nevertheless, TB remains a major global health threat. The national health TB situation in Slovakia is as yet unchanged, with successful treatment outcomes reaching over 90% of cases. Low housing quality, unfavourable social background, and abandonment of anti-TB vaccination efforts could contribute to newly detected cases. Furthermore, the increasing migration rate due to the ongoing war in Eastern Europe could potentially affect the incidence of the disease. First-line antituberculotics represented by parenteral streptomycin and oral forms of isoniazid, ethambutol, pyrazinamide, rifampicin, and derivatives rifabutine and rifapentine hold an exclusive position in the drug-susceptible TB treatment regime. Therapeutic drug monitoring (TDM) is a measurement of plasma drug concentrations. It is directly linked with the individualisation of pharmacotherapy by maintaining plasma levels of the drugs within the therapeutic range. Because TDM is improving the clinical potential of therapeutics and simultaneously lowering toxicity risk, it passes the assessment of efficacy and safety. Despite many advantages of TDM, TB treatment requires further investigation on outcomes improvement. Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) offers high-quality measured data, as it has adopted enhanced analytical specificity and high sensitivity over conventional immunoassay methods. A new LC-MS/MS method for simultaneous detection and quantification of isoniazid, pyrazinamide, and ethambutol was developed and fully validated according to the European Medicine Agency guidelines. Determining drug plasma levels enables clinicians to achieve the targeted serum concentrations required for successful clinical and bacteriological outcomes.

Keywords *antituberculotics – mass spectrometry – tuberculosis*

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Inhibition of O-6-methylguanine-DNA methyltransferase in Vascular Smooth Muscle Cells Leads to Activation of mTORC1 Signalling Pathway and Inhibition of FOXO3a Protein

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Abstract Cellular senescence represents an irreversible state of cell cycle arrest that can be triggered by various factors, such as DNA damage. Accumulation of senescent cells with age contributes to the development of age-related diseases including atherosclerosis. O^6-methylguanine DNA methyl*transferase (MGMT) removes alkyl residues from guanine on DNA*. O^6-benzylguanine (BG) is a potent inhibitor of MGMT and its use has been proposed in treatment of cancer together with alkylating agents. Previously we have shown that inhibition of MGMT by BG causes senescence on vascular smooth muscle cells (VSMCs). We decided to investigate molecular mechanisms that lead to cellular senescence caused by MGMT inhibition. The aim of our work was to investigate whether the mTOR pathway and FOXO3a are involved in the development of VSMCs' senescence. VSMCs were isolated from rat aorta and cultured with BG for 72 hr at a concentration of 100 μM. Subsequently we used SDS-PAGE and western blotting to analyse the expression of $p27^{Kip1}$, mTOR, p-mTOR Ser2448, p-mTOR Ser2481, Raptor, Rictor, GβL, and FOXO3a. We confirmed VSMCs' senescence by significantly increased expression of $p27^{Kip1}$. mTOR complex 1 (mTORC1) contains mTOR Ser2448, Raptor, and GβL and mTOR complex 2 (mTORC2) contains mTOR Ser2481 and Rictor. Expression of mTOR and proteins involved in mTORC1 was significantly increased in BG-treated cells and expression of transcription factor FOXO3a was significantly decreased. In the case of proteins in mTORC2, the expression was not changed. These results suggest that mTORC1 was activated, but mTORC2 was not affected by the effect of BG. FOXO3a, which is negatively regulated by mTORC1, was found to be decreased. We confirmed that BG-induced senescence activated mTORC1, but not mTORC2, and decreased expression of FOXO3a, which might contribute to the development of cellular senescence. Further investigation could help to elucidate the role of mTORC1 and FOXO3a in cellular senescence induced by MGMT inhibition and could lead to an understanding of the onset of adverse effects of BG on the cardiovascular system after cancer treatment.

Keywords *cellular senescence – O^6-metylguanine DNA methyltransferase – O^6-benzylguanine, mTORC1, FOXO3a*

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Lymphatic Absorption of Cannabidiol: First in vivo Study in Rats

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Abstract Cannabidiol (CBD) is a natural alkaloid present in *Cannabis sativa*. It is used in the treatment of specific types of epilepsy and a wide range of other possible indications are currently under investigation. CBD is a highly lipophilic drug (logP 6.3). It has been previously shown that drugs with logP > 5 have a potential of significant lymphatic transport through mesenteric lymph vessels on oral administration. This type of absorption could be of great pharmacological importance because it reduces the liver first-pass elimination and brings the active substance into the intestinal lymphatic system, where the CBD immunomodulation effect can come into play. We used our anaesthetised lymph duct cannulated rat model and quantified the extent of CBD lymphatic transport. Additionally, standard pharmacokinetic studies comparing oral bioavailability of two CBD formulations (microemulsion and sunflower oil solution) were conducted. In the results, CBD oral bioavailability was 25% for microemulsion and 14% for sunflower oil solution. CBD lymph concentrations were two to three orders of magnitude higher than serum concentrations. Exactly quantified, 39% and 55% of systemically available active substance has been found in the mesenteric lymph after microemulsion and sunflower oil solution administration, respectively. In conclusion, CBD shows moderate to low bioavailability after oral administration. On the other hand, lymphatic transport plays a crucial role in the process of intestinal absorption and distribution.

Keywords *pharmacokinetics – bioavailability – lymph duct cannulation – oral administration – intravenous administration*

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Covariates for Ganciclovir Pharmacokinetics in Lung Transplant Recipients as a Tool for Dosing Optimisation

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Abstract Although ganciclovir is routinely used in lung transplant recipients, its dosing individualisation has not yet been clearly addressed. Therefore, the aim of this prospective study was to evaluate the pharmacokinetics of ganciclovir in lung transplant recipients and to explore its covariates to propose an individualised ganciclovir dosing regimen prior to therapeutic drug monitoring (TDM). Ganciclovir was administered in a standardised intravenous dose of 5 mg/kg every 12 hr. Ganciclovir serum levels were monitored as a trough and at 3 hr and 5 hr after the infusion was completed. Individual pharmacokinetic parameters of ganciclovir were calculated in a two-compartmental pharmacokinetic model and regression models were applied to explore the covariates. Optimal loading and maintenance doses were simulated for each patient. In 40 lung transplant recipients, the median (IQR) ganciclovir total volume of distribution (Vd) and clearance (CL) values were 0.65 (0.52–0.73) L/kg and 0.088 (0.059–0.118) L/hr/kg, respectively. We observed medium to high interindividual but negligible intraindividual variability in ganciclovir pharmacokinetics. Both Vd and CL normalised by body weight were significantly and negatively related to age. Males showed significantly higher body weight-normalised Vd than females. Vd of central compartment was significantly related to body weight, height, BSA and body mass index, whereas total Vd increased significantly only with height and BSA. CL was significantly related only to eGFR. Ganciclovir exposure was not associated with either white blood cell or platelet counts. Body weight-normalised CL was significantly higher in patients with cystic fibrosis and there was also a trend toward increased volume of distribution in these patients. We observed no drug interaction between immunosuppressive or antimycotic therapy and ganciclovir weight-normalised pharmacokinetic parameters. On the basis of observed relationships, easy-to-use nomograms for individualised ganciclovir dosing were constructed. Dosing of ganciclovir in patients with cystic fibrosis requires special caution, as their daily maintenance dose should be increased by approximately 50%.

Keywords *ganciclovir – therapeutic drug monitoring – covariates – lung transplant recipients – cystic fibrosis*

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Coffee Consumption and Atherosclerosis: Risk or Protective Factor?

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Abstract Since the very beginning of the Framingham Trial, there has been ongoing debate on the impact of coffee consumption on atherogenesis and coronary heart disease. Various retrospective studies have reported inconsistent results about an association between coffee consumption and risk of atherosclerosis. Recent meta-analysis of observational cohort studies confirmed the protective effect of coffee drinking on all-cause mortality, cardiovascular mortality, heart failure, and sudden death, but not on major cardiovascular and cerebrovascular events. Deleterious effects of caffeine on myocardial perfusion were observed in a study of healthy volunteers undergoing PET and CT scans. However, there is little information on the effect of coffee consumption on endothelial dysfunction. It is expected that coffee and caffeine exert beneficial effects on endothelial function through enhancement of NO bioavailability. There are a few papers published indicating coffee drinking could also influence parasympathetic activity related to heart rate variability. On the other hand, acute administration of caffeine elevates blood pressure due to elevation of peripheral vascular resistance rather than an increase of cardiac output. The vasoconstrictor potential of caffeine is probably mediated through several mechanisms leading to increased sympathetic activity. It is not clear how a balance between caffeine-induced augmentation of endothelial function and caffeine-induced blood pressure elevation affects the progression of atherosclerosis and cardiovascular morbidity and mortality. Moreover, coffee intake might have an additional antiatherogenic property by improving reverse cholesterol transport by increase of ABCG1 and SR-B1 expression and enhancing HDL mediated efflux from macrophages via its plasma phenolic acids.

Keywords *coffee – caffeine – atherosclerosis – endothelial function – cardiovascular morbidity*

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Role of Dipeptidylpeptidase-4 in the Development of Heart Damage

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Abstract Several animal models have indicated a cardioprotective effect of inhibition of dipeptidylpeptidase-4 (Dpp-4), which might be caused by inhibition of catalysis of cardioprotective substrates. However, the expression of Dpp-4 in relation to cardiac damage due to different pathological mechanisms has not been sufficiently studied. We analysed the mRNA expression of Dpp-4 and selected markers of cardiac damage in various rat models. We hypothesised that the cardiac damage might be associated with upregulation of Dpp-4, thus contributing to increased degradation of cardioprotective substrates. Diabetic cardiomyopathy was induced by single administration of streptozotocin (50 mg/kg i.p., 5 days before asphyxiation). Anthracycline cardiomyopathy was induced by daunorubicin (acute model, 6 × 3 mg/kg i.p., every 48 hr; chronic model, 15 mg/kg i.v. single dose 8 weeks before asphyxiation). Isoproterenol (5 mg/kg/day i.p.) was administered to rats for 8 days. Pulmonary hypertension was induced by a single dose of monocrotaline (60 mg/kg s.c.). The last experimental model was induced by testosterone (100 mg/kg s.c. once a week for 8 weeks). All models included vehicle treated control groups. Dpp-4 was downregulated in all models studied: streptozotocin (–59% vs. control group), acute daunorubicin cardiomyopathy (–28%), chronic (–56%), isoproterenol cardiomyopathy (–28%), monocrotaline pulmonary hypertension (–32% for advanced stage, –58% for terminal stage), and testosterone model (–35%), all $p < .05$. Cardiac damage markers were changed in almost all models, except for the acute daunorubicin and testosterone models. Expression of Dpp-4 correlated with expression of cardiac troponins. Unexpected downregulation of Dpp-4 expression might compensate for the development of cardiac damage by decreased degradation of cardioprotective substrates. However, the compensation is probably insufficient in the advanced stages of heart damage.

Keywords *cardiac damage markers – dipeptidylpeptidase-4 – diabetes*

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