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MEETING ABSTRACTS

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*PUBLISHED MEETING ABSTRACTS**

Oral Presentations

A1.1

The role of the molecular circadian clock in asthma

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Background: Asthma is a chronic inflammatory lung disease with a strong circadian signature. In 75% of all patients, symptoms worsen overnight; most severe asthma attacks occur at 4 a.m., and the highest number of eosinophils, one of the main effector cells in asthma, is observed in the sputum at this time. Many biological processes such as leukocyte trafficking are controlled by the molecular circadian clock which is driven by interacting feedback loops. As disturbances within the circadian system promotes inflammatory diseases, this project aims to investigate the impact of the molecular circadian clock in asthma. Synthetic compounds such as the RAR-related orphan receptor (ROR) inverse agonist SR1001 target nuclear receptors of the circadian feedback loops pharmacologically, and hence might represent a novel treatment approach in the future.
Methods: Whole blood or isolated immune cells were used for *ex vivo* experiments including chemotaxis, respiratory burst or degranulation assays, or to culture human monocyte-derived macrophages. To investigate the circadian responsiveness, cells were incubated with sera from asthmatic and healthy donors, stimulated with pro- or anti-inflammatory mediators or treated with the ROR inverse agonist SR1001. To study the systematic effect of SR1001 *in vivo*, a murine eosinophil migration model was used. Additionally, the LabMaster system was employed to analyse the effect of pharmacologically targeting the molecular circadian clock on behaviour pattern of the mice.
Results: An oscillating expression of nuclear circadian receptors of the accessory loop in leukocyte subsets including eosinophils, neutrophils, T cells and monocytes was observed by a whole-blood-staining experiment. Comparing expression levels of these circadian receptors from asthmatic and healthy donors, significant differences in distinct components were revealed depending on the time of the day. Similar differences were observed in human polymorphonuclear leukocytes (PMNLs) in a mimicked inflammatory environment using asthmatic sera or pro-inflammatory mediators. The ROR inverse agonist SR1001 reduces the shape, the migratory responsiveness, respiratory burst and the degranulation of human peripheral PMNLs. SR1001 treatment also affects the nuclear circadian receptor expression and polarization of macrophages. Further, systemically applied SR1001 reduces the number of migrated eosinophils in the BAL fluid of IL-5 transgenic mice. Importantly, targeting the accessory loop using SR1001 had no effect on the physiological pattern of activity, drinking or eating rhythm.
Discussion: We observed that the molecular circadian clock oscillates on the receptor level in human leukocytes and is altered under inflammatory conditions such as asthma. Targeting the ROR receptor has an impact on the expression level of the circadian receptors and supresses effector cell functions of eosinophils, neutrophils and macrophages. Further, SR1001 shows anti-inflammatory properties *in vivo,* reducing the migratory responsiveness of eosinophils. Thus, the exogenously applied ROR inverse agonist SR1001 may represent a novel pharmacological approach for the treatment of allergic inflammation and asthma.
Keywords: asthma – molecular circadian clock – inflammation – RAR-related orphan receptor inverse agonists – SR1001

A1.2

Pharmacological modulation of TPC1 regulates inter-organellar Ca^{2+} homeostasis in immune cells and plays an important role in allergic hypersensitivity

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Background: Mast cells and basophil granulocytes are important for anaphylaxis and allergic reactions by releasing inflammatory mediators such as histamine and heparin. The cytoplasm of these innate immune cells contains a large number of granules comprising these messenger substances. We recently linked the endolysosomal two-pore channel TPC1 to systemic anaphylaxis *in vivo* and underlying mast-cell function *ex vivo* [1]. Mice, in which TPC1 was genetically or pharmacologically inhibited, developed increased systemic anaphylaxis. Inhibition of TPC1 enhanced mast-cell degranulation and concomitant histamine release due to accelerated Ca^{2+} release.

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Signalling in mast cells is mainly regulated by the release of Ca^{2+} from the endoplasmic reticulum (ER), but also from acidic compartments such as endolysosomes. The stimulation of TPC1 by endogenous ligands such as nicotine adenine dinucleotide phosphate (NAADP) or phosphatidylinositol 3,5-bisphosphate ($PI(3,5)P_2$) triggered the release of Ca^{2+} from endolysosomes. This enhanced the effect of TPC1 in regulating mast-cell degranulation. Apart from these important insights into TPCs physiology [2], there is a lack of knowledge about the underlying ultrastructural processes.

Methods: We have therefore implemented 2D and 3D transmission electron microscopic (TEM) methods to investigate the ultrastructure of rat basophilic leukemia cells (RBL-1), treated with or without the plant alkaloid and TPC inhibitor tetrandrine.

Results: Our 2D TEM investigations with RBL-1 controls showed that ER and endolysosomes formed inter-organellar contact sites. Moreover, 3D TEM tomography revealed the full extent of the large contact surfaces between the two organelles. In comparison, these contact surfaces significantly decreased in cells treated with tetrandrine, further supporting the hypothesis that TPC function is essential for inter-organellar Ca^{2+} exchange.

Discussion: Here, we aim at a better understanding of the role of TPC channels in the regulation of the crosstalk between ER and endolysosomes at an ultrastructural level. Correlating our physiological and ultrastructural findings with analytical EM, as well as with further Ca^{2+} imaging, molecular biological and immunological experiments could help clarify whether TPC channels are indeed promising pharmacological targets for the treatment of allergic hypersensitivity.

Keywords: two-pore channels – TPC1 – anaphylaxis – mast cells – calcium homeostasis – electron microscopy

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A1.3

TRPC6 photopharmacology enables therapeutic modulation of the mast-cell signaling signature

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Background: Mast cells release a wide range of potent inflammatory mediators that give rise to these immune cells' complex role within the tumor microenvironment. Release of pre-stored mediators is likely to promote tumor progression, while nuclear factor of activated T cells (NFAT) transcriptional activation in mast cells leads to the *de novo* synthesis of potentially beneficial, antitumor mediators. Mast-cell degranulation involves a heterogenous set of cellular mechanisms, and to date specific interference, especially interventions that dissect Ca^{2+}-mediated exocytosis from Ca^{2+} transcription coupling in mast cells are lacking. Here, we tested a novel optochemogenetic approach, which targets TRPC6 Ca^{2+} channels, for suitability to specifically tailor the immunological signature of mast cells.

Methods: All experiments were performed using rat basophilic leukaemia cells (RBL-2H3) cells, genetically modified to overexpress TRPC6 channels along with a reporter of NFAT translocation and CD63 as a standard marker for immune (mast)-cell degranulation. Intracellular Ca^{2+} changes were monitored by fluorescence imaging using Fluo-4 AM.

Results: Genetic manipulation of RBL cells to overexpress TRPC6 conferred susceptibility to photopharmacological control of temporal features of Ca^{2+} signaling. Exposure of cells to the inactive *trans* isomer of the photochromic benzimidazole OptoBI-1, failed to induce degranulation and/or NFAT transcriptional activity. Repetitive photoactivation of OptoBI-1 generated oscillatory Ca^{2+} signals associated with rapid and efficient NFAT nuclear translocation. Importantly, OptoBI-1-mediated oscillatory Ca^{2+} signals introduced by a sequence of short (15 s) light pulses generated robust NFAT activation without detectable degranulation of the mast cells.

Discussion: Our results demonstrate that TRPC6/OptoBI-1-based optical modulation of mast-cell Ca^{2+} signals allows for unprecedented specificity in targeting mast-cell functions. The described optochemogenetic approach was found suitable to initiate specific transcriptional activation of the NFAT pathway coupled with the generation of a potentially antitumor immunological signature. Our results provide evidence for a potential therapeutic value of (photo)pharmacological strategies for high-precision immunomodulation in cancer therapy.

Keywords: TRPC6 – photopharmacology – mast-cell degranulation – NFAT

A1.4

The role of biliverdin reductase in the protective activity of bilirubin in human endothelium

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Background: Bilirubin is an endogenous antioxidant with anti-inflammatory and anti-thrombogenic activity. Recent studies showed that bilirubin is an essential antioxidant in the human endothelium. The current study aimed to determine the role of biliverdin reductase in the protective activity of bilirubin in the human endothelium.

Methods: Our experiments were done on the human endothelial cell line EA.hy926. Cells were exposed for 12 h to simulated oxidative stress conditions using the peroxyl radical initiator 2,2'-azobis(2-amidinopropane) dihydrochloride (ABAP). We studied the protective activity of bilirubin in the physiological concentration range of free unbound bilirubin (10–100 nM). To examine the role of BVR activity, we simultaneously incubated the endothelial cells with the BVR inhibitor apomorphine (10 nM). After 12 hours (at the end of oxidative stress), we determined the intracellular antioxidant activity of bilirubin using a CAA assay. We also measured the endothelial cell viability by resazurin-based fluorescent assay. To determine the cellular levels of bilirubin and biliverdin, we scraped and collected endothelial cells into methanol-DMSO solution, and analyzed them using HPLC-MS.

Results: Our experimental data confirmed that bilirubin acts as a strong intracellular antioxidant, and thus ameliorated the simulated oxidative-stress-induced injury. We discovered that inhibition by apomorphine of BVR decreased endothelial cell viability, decreased

intracellular antioxidant activity, and lowered cellular levels of bilirubin.

Discussion: Our results suggest that BVR plays an essential role in bilirubin homeostasis in the human endothelium under increased oxidative stress. Endothelium exposed to high levels of ROS oxidizes more bilirubin to biliverdin, and then BVR plays an essential role in recycling biliverdin back to bilirubin. Our main finding is that inhibition of BVR diminished the protective activity of bilirubin on endothelial cells.

Acknowledgements: This work was financially supported by the Slovenian Research Agency (research program P3-0067: Pharmacology and Pharmacogenomics).

Keywords: bilirubin – biliverdin – biliverdin reductase – endothelium – oxidative stress

A1.5

The anti-Warburg ruthenium compound BOLD-100 targets the cellular lipid metabolism and impacts on histone acetylation

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Background: The ruthenium anticancer complex sodium *trans*-tetrachloro-[bis(1*H*-indazole)ruthenate(III)] (BOLD-100) currently undergoes multicentre clinical phase II evaluation in combination with the FOLFOX regimen against several solid tumor types. Systemically, BOLD-100 hitchhikes serum albumin and, thereby, accumulates in the malignant tissue where it is 'activated by reduction'. Mechanistically, BOLD-100 is an ER stress inducer via the inhibition of the chaperone glucose-regulated protein 78 (GRP78) leading to unfolded protein response and caspase-dependent apoptosis. A major limitation for effective cancer therapy represents the acquisition of resistance. Thus, the dissection of underlying mechanisms is necessary already during (pre)clinical development.

Methods: In this study, colon cancer HCT 116 and pancreatic cancer Capan-1 cells were selected for BOLD-100 resistance. Cell/molecular biological as well as analytical chemistry methods and omics approaches—including, amongst others, metabolomics, transcriptomics, Seahorse XF analyses, western blotting, cell viability assays, mass spectrometric (MS) and nuclear magnetic resonance (NMR) analyses—were used to dissect molecular mechanisms underlying acquired BOLD-100 resistance.

Results: BOLD-100 was identified as anti-Warburg drug reducing lactate and pyruvate levels of parental HCT 116 cells. Computational network analysis of gene expression and metabolomics data revealed a clear upregulation of glycolysis in BOLD-100-resistant HCT 116 (HCTR) cells associated with increased pyruvate and citrate levels creating a vulnerability towards glucose deprivation by 2-deoxy-D-glucose (2-DG). The enhanced glycolytic activity fueled into cellular lipid enrichment. Accordingly, increased lipid droplet (LD) levels in HCTR cells were associated with an altered *de novo* lipid synthesis. Hence, pharmacological targeting of the lipid phenotype with diverse lipid metabolism inhibitors, such as triacsin C or the β-oxidation inhibitor etomoxir, exposed the lipid metabolism as Achilles' heel of cells and xenograft models with acquired BOLD-100 resistance. Coenzyme A (CoA), as key metabolite, connects glycolysis via the tricarboxylic acid (TCA) cycle with the lipid metabolism and, consequently, histone acetylation. BOLD-100 treatment reduced histone acetylation only in parental HCT 116 cells while it enhanced this parameter in HCTR cells. Thus, a potential interaction between BOLD-100 and CoA was postulated. Consequently, BOLD-100 and CoA were co-incubated under cell-free conditions and, indeed, MS and NMR analyses revealed the formation of a BOLD-100-CoA thioester. Upon combination of BOLD-100 with another CoA-binding compound, namely 4-phenylbutyric acid (PBA), synergistic anticancer activity in several tested cancer models and, furthermore, the reversal of acquired BOLD-100 resistance were observed.

Discussion: In summary, the strong metabolic activity of BOLD-100 identified in this work presents a novel mode of action of the ruthenium complex. This interference with several hubs of the complex onco-metabolism network bears huge potential for broad therapeutic applicability. Current work focusses on further in-depth investigation of the specific crosstalk between diverse cellular metabolic routes and the identification of synergism-associated mechanisms of reasonable therapeutic combination partners.

Acknowledgements: We wish to thank the Austrian Science Fund FWF, the Fellinger Cancer Research Fund and the Mahlke-Obermann Foundation for their financial support.

Keywords: BOLD-100 – ruthenium-based compounds – cancer chemotherapy resistance – glycolysis – lipid metabolism – histone acetylation

A1.6

PET-imaging-based pharmacokinetic analysis to assess the influence of transporters on the tissue distribution and renal excretion of ciprofloxacin

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Background: Ciprofloxacin is a commonly prescribed antibiotic which has been associated with severe side effects such as central nervous system toxicity. Ciprofloxacin is mainly excreted in unchanged form into the urine. Due to its zwitterionic nature and poor membrane permeability, the organ distribution of ciprofloxacin largely depends on the activity of both cation and anion transporters, which are expressed at the basolateral and apical membranes of kidney proximal tubule cells and govern tubular secretion of many drugs. Therefore, ciprofloxacin is susceptible to transporter-mediated drug–drug interactions (DDI), which may lead to changes in ciprofloxacin plasma and tissue pharmacokinetics (PK) and may exacerbate its side effects. In this study, we used positron emission tomography (PET) imaging in mice to assess the effect of two drugs,

which are known to be involved in transporter-mediated DDIs with ciprofloxacin (probenecid and cimetidine), on the tissue distribution and excretion of [^{18}F]ciprofloxacin.

Methods: Mice underwent dynamic PET scans after i.v. administration of [^{18}F]ciprofloxacin without and with pre-treatment with either probenecid (150 mg/kg) or cimetidine (50 mg/kg). From the PET data, time–activity curves (TACs) were extracted for blood (image-derived blood curve from the left ventricle of the heart), kidneys, urinary bladder, lungs and brain. The area under the TACs (*AUC*) for each organ was calculated as a measure of ciprofloxacin exposure. The total renal clearance ($CL_{urine, blood}$) of [^{18}F]ciprofloxacin was calculated. A previously developed 3-compartment PK model was used for a detailed assessment of the effect of transporters on the renal disposition of [^{18}F]ciprofloxacin.

Results: $CL_{urine, blood}$ was significantly decreased after pre-treatment with both probenecid (2.7-fold) and cimetidine (7-fold) as compared to untreated mice. PK modelling revealed pronounced decreases in the uptake of [^{18}F]ciprofloxacin from blood into the kidneys (CL_1) after both probenecid (2.4-fold) and cimetidine (5.7-fold) pre-treatment. In addition, the transfer of [^{18}F]ciprofloxacin from the kidneys into urine (k_3) was significantly reduced after pre-treatment with probenecid (6.2-fold) and cimetidine (693-fold). Changes in the urinary excretion of [^{18}F]ciprofloxacin after pre-treatment with both inhibitors resulted in increases in blood exposure (AUC_{blood}: 1.3- and 1.8-fold for probenecid and cimetidine, respectively), which in turn led to similar increases in brain (AUC_{brain}) and lung (AUC_{lung}) exposure to [^{18}F]ciprofloxacin.

Discussion: Our results suggest that ciprofloxacin is taken up into the kidneys and excreted into urine by transporters, *e.g.* OAT3/SLC22A8 in the basolateral membrane and MATE1/SLC47A1 and MATE2-K/SLC47A2 in the apical membrane. Concomitant medication that inhibits transporter activity in kidney proximal tubule cells can cause DDIs, leading to decreases in the urinary excretion of ciprofloxacin. This may increase blood and organ exposure to ciprofloxacin and thereby potentially exacerbate its adverse effects. Our study highlights the strength of PET-imaging-based PK analysis to untangle individual mechanisms that are relevant for transporter-mediated drug excretion such as basolateral uptake, basolateral efflux and apical efflux processes. The upcoming availability of total-body PET systems in combination with this analytical approach bears great potential for translation to humans to quantitatively assess drug disposition and DDIs on a whole-body level.

Keywords: ciprofloxacin – membrane transporters – PET imaging – PK modelling – renal excretion

A1.7

Effect of a previous COVID-19 infection on the retinal vasculature

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Background: Since March 2020, the COVID-19 pandemic has affected countries around the world and continues to challenge humanity. The most common symptoms during infection are respiratory phenomena. However, there is also increasing evidence of neurological and vascular symptoms as the disease is associated with CNS manifestations and endothelial dysfunction. Whether residuals remain after patients have recovered from COVID-19 infection is still the subject of current research. We set out to investigate whether ocular vascular alterations remain after recovery from COVID-19 infection.

Methods: In the present study, we included patients previously infected with COVID-19 and healthy age- and sex-matched controls. Patients needed a confirmed positive PCR test for SARS-CoV-2 infection in the medical history within the last 6 months and positive testing for SARS-CoV-2 seroprevalence for inclusion. Negative testing for SARS-CoV-2 seroprevalence and no history of COVID-19 infection were inclusion criteria for controls. A dynamic vessel analyzer (DVA, Imedos, Germany) was used to measure arterio-venous (AV) difference in oxygen saturation, retinal vessel diameters, and arteriovenous ratio (AVR). AV was calculated out of retinal arterial and venous oxygen saturation. We used laser speckle flowgraphy (LSFG, Nidek, Japan) to additionally quantify the mean blur rate in the tissue area of the optic nerve head (MT).

Results: Twenty-nine patients who had recovered from moderate-to-severe COVID-19 infection requiring hospitalization (mean age 35 ± 17 years) and 11 control subjects (mean age 36 ± 12 years) met our inclusion criteria. We found no differences between the two groups in terms of sex or concomitant diseases in the medical history. Patients had a significantly higher body mass index (BMI) than healthy controls (27.5 ± 5.6 *vs.* 24.5 ± 2.8 m^2/kg, $p = 0.036$). Compared to control subjects both AVR and AV difference in oxygen saturation were significantly lower in patients ($p = 0.021$ for AVR and $p = 0.023$ for AV difference in oxygen saturation). It was also shown that MT in the optic nerve head was significantly lower in patients (23.4 ± 10.1 a.u.) than in healthy controls (47.3 ± 26.6 a.u., $p < 0.001$).

Discussion: This study shows changes in retinal vessels as well as in optic nerve head blood flow in patients who have recovered from COVID-19 infection. Further, longitudinal studies are required to investigate whether these changes in retinal metabolism are temporary or permanent.

Keywords: COVID-19 – ocular blood flow – retinal oxygen saturation

A1.8

The impact of the COVID-19 pandemic on the consumption of anxiolytic drugs in Serbia: an analysis for the period 2019–2021

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Background: The consumption of psychotropic drugs shows a constant growth trend and WHO recognizes it as a global phenomenon. Benzodiazepine (BZD) anxiolytics have been among the most prescribed medications in clinical practice for decades for their anxiolytic, sedative-hypnotic, muscle-relaxant and anticonvulsant properties. However, their prescription is still insufficiently regulated in some countries, including Serbia. According to current recommendations, there is a wide spectrum of BZD use in psychiatric clinical practice, such as anxiety and affective disorders, alcohol withdrawal, sleep disorders, delirium, aggressive behaviour in psychoses and neuroleptic-induced disorders. However, the development of tolerance and dependence after long-term treatment with BDZs, as well as the possibility of abuse, limit their use. A task force of the World Psychiatric Association suggests that they should not be

prescribed for periods longer than 30 days, followed by a gradual dose reduction, except for chronic or recurrent disorders.

Methods: This study aimed to analyse mental health care in Serbia, with a focus on the anxiolytic drug consumption among the population. Two data sources were analysed for the period 2019–2021: Agency for Medicines and Medical Devices of Serbia (ALIMS) and Intercontinental Medical Statistics (IMS Health). The ATC/DDD methodology, recommended by the WHO, was used to analyse drug consumption for the three most used oral benzodiazepines: diazepam, bromazepam and alprazolam. The number of consumed defined daily doses (DDD) per 1000 inhabitants per day was taken as indicator of drug consumption.

Results: There was a significant increase in BZD consumption in 2020 and 2021. Diazepam, as a long-acting, moderately strong BZD, was the most used compound (up to 20% increase during the pandemic), recently replaced by bromazepam. During the current pandemic, alprazolam had the most significant increase in consumption of all other BZDs (up to 25% increase). The total consumption of BZDs in Serbia is approximately 2.5 times higher as compared to the EU. In contrast, in Serbia the consumption of antidepressants (SSRIs) is still significantly lower than in EU countries. Short-acting benzodiazepines, such as alprazolam, have the greatest potential for addiction and are surprisingly the most widely used anxiolytics. The highest rate of BZD use was found in the elderly population, which is particularly vulnerable to the BZD side effects, such as fractures, falls and cognitive deficits.

Discussion: The results of our study indicate a potential issue of excessive use (misuse/abuse) of BZDs followed by reduced use of antidepressants in Serbia, which can be partially explained by poor access to mental health care during the pandemic. According to data from the UK, alprazolam is increasingly used by the younger population for non-medical purposes, while according to European data, Serbia and Croatia are among the countries with the highest rate of diazepam consumption, especially in the elderly population. Therefore, it is urgently necessary to identify all the factors that influence the excessive use of these drugs, and consequently to revise the pharmacotherapeutic approaches and attitudes in clinical practice [1]. The COVID-19 pandemic has a significant impact on BZD consumption, indirectly reflecting the state of the population's mental health.

Keywords: anxiolytics – benzodiazepines – COVID-19 – drug utilization – pharmacoepidemiology

Reference

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A1.9

Predicting the future – How accurate are the sales forecasts in Austrian reimbursement applications?

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Background: In Austria, applications for listing outpatient medicines for reimbursement must include forecasts of future sales, to inform payers of potential budget impact. The accuracy of these forecasts has not been evaluated.

Methods: The data presented here are based on a retrospective, systematic analysis of 102 applications for new drugs, which were successfully submitted between 2005 and 2014 and for which reimbursement data were available for at least 3 years. The accuracy ratio was defined as the ratio of forecasted sales submitted by pharmaceutical companies in the reimbursement application *versus* actual sales from administrative data. All analyses were performed using R 3.6.3.

Results: The median accuracy ratio was 1.33 (95% confidence interval 1.03–1.74, range 0.15–37.5). Thus, more than half of all forecasts were overestimates by 33% or more. For 37.3% of all examined products, forecasts severely overestimated actual sales by more than 100%. Severe underestimation by more than 50% was seen for 18.6% of all products. Reimbursement status (restricted or unrestricted), the degree of therapeutic benefit, or the therapeutic area of the pharmaceutical product did not discernibly influence accuracy, nor were any systematic changes observed over time.

Discussion: The majority of submitted forecasts was highly inaccurate and on average overestimated sales and thus budget impact. This phenomenon has also been observed in other jurisdictions and estimates from other institutions. If these forecasts were to be useful for estimating the budget impact of new pharmaceuticals, their accuracy needs to be improved.

Acknowledgements: We thank Gerald Hlavin for his support in the data analysis.

Keywords: budget impact – forecasting accuracy – reimbursement of pharmaceuticals

A1.10

Myeloperoxidase alters tumor growth *in vitro* and *in vivo*

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Background: Despite the recent improvements in treatment, lung cancer remains the leading cause of cancer-related deaths worldwide. Non-small-cell lung cancer (NSCLC) is a heterogeneous disease and represents ~85% of all lung cancer cases. It has been reported that NSCLC tumors exhibit high immune-cell infiltration, including high neutrophil infiltration. Neutrophil-derived enzymes, such as myeloperoxidase (MPO), are considered to contribute to tumor development. MPO is a heme-containing peroxidase enzyme known for its host-defence function against microbes. Some reports suggest that MPO might be able to influence cancer cells and the tumor microenvironment and that way contribute to cancer development. We aim to investigate whether MPO can influence tumor growth *in vitro* and *in vivo*.

Methods: For this investigation we used, among others, the following methods: flow cytometry, fluorescence microscopy, western blotting, and BrdU, FITC annexin V/PI and wound-healing assays.

Results: *In vivo* data in our lab show that MPO knockout (KO) mice develop smaller tumors and have prolongated survival when compared to MPO wildtype (WT) mice. Analysis of the tumor microenvironment (TME) revealed an increased number of different T-cell populations as well as improved function of T cells in MPO KO *vs*. WT mice. Further, MPO increased proliferation of human lung adenocarcinoma A549 cells *in vitro*. Furthermore, MPO-treated cells revealed a decreased number of apoptotic cells, suggesting a protective function of MPO. Migration behaviour of cancer cells was

not affected by MPO. Besides the cytoplasmic uptake of MPO, we report for the first time a nuclear internalization of MPO in A549 cells.
Discussion: Our data support the hypothesis that MPO plays a role in lung cancer development.
Keywords: lung cancer – myeloperoxidase – neutrophils

Poster Presentations

A2.1

The balance between STAT3 isoforms as essential feature in acute myeloid leukemia

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Background: Dysregulation of the JAK/STAT pathway and overexpression of the signal transducer and activator of transcription 3 (STAT3) is frequently found in hematologic malignancies. Constitutive activation of STAT3 in leukemic blasts of patients is associated with significantly poorer outcomes. As a consequence, STAT3 became an attractive therapeutic target. However, until now, drugs targeting STAT3 have not led to the intended effects. This might be related to the expression ratio of the two alternatively spliced isoforms, the full-length isoform STAT3α and the C-terminally truncated STAT3β. Recently, our research group has shown that STAT3β acts as a tumor suppressor in acute myeloid leukemia (AML). In line, the *STAT3β/α* mRNA ratio in leukemic blasts of patients with bad prognosis was found to be significantly lower than in those from patients with good prognosis. In the light of these previous findings, the pharmacological induction of a higher STAT3β/α ratio could be a novel therapeutic option in AML.
Methods: To examine the efficiency of candidate drugs in affecting the STAT3 isoform ratio in leukemic cells, we performed several *in vitro* assays such as real-time quantitative PCR and western blot. Moreover, to control for specificity of the effect on the isoform ratio change, we use AML cell lines with STAT3β overexpression or knockout. In addition, we plan to test the anti-leukemic drug activity *in vivo*, using an MLL/AF9-induced mouse model and xenograft models.
Results: Our current data identified the common antimalaria drug atovaquone as a potentially attractive candidate. We determined that the treatment with atovaquone increases the STAT3β/α ratio on protein and mRNA level in different human and murine AML cell lines. The effect is paralleled by an anti-leukemic activity of atovaquone. Using AML cell lines with STAT3β overexpression or knockout, we are currently testing the exact mechanism of atovaquone-induced effects. Furthermore, we plan to address the anti-leukemic effect of atovaquone *in vivo*.
Discussion: We have demonstrated a novel property of the antimalaria drug atovaquone. It positively affects the STAT3β/α ratio in AML cell lines, which could be potentially of therapeutic relevance in AML.
Keywords: acute myeloid leukemia – STAT3 – atoquavone

A2.2

STAC proteins inhibit calcium- and voltage-dependent inactivation of L-type calcium channels

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Background: Recently, we demonstrated that calcium-dependent inactivation (CDI) of L-type calcium channels (LTCC), an important negative feedback mechanism in calcium signaling, is inhibited by STAC proteins. This could be demonstrated for $Ca_V1.2$, $Ca_V1.3$ and $Ca_V1.4$, but not for the skeletal muscle channel $Ca_V1.1$, as it requires STAC3 for its functional expression. Interestingly, $Ca_V1.1$ currents show negligible inactivation, which could be either an intrinsic property of the channel or the result of an inhibitory effect of STAC3 on the inactivation of $Ca_V1.1$.
Methods: In order to discriminate between these two possibilities, we inserted a triple mutation in the linker region of STAC3 (ETLAAA), as the analogous mutation in the paralog STAC2 was shown to abolish the inhibitory effect on the CDI of $Ca_V1.3$. We performed patch-clamp electrophysiology experiments in our GLT $Ca_V1.1$/STAC3 double knockout cell line, or HEK cells with either calcium or barium in the extracellular solution to distinguish between CDI and VDI (voltage-dependent inactivation).
Results: In patch-clamp electrophysiology experiments in myotubes, we found that STAC3-ETLAAA coexpression results in dramatically faster kinetics of activation and inactivation of $Ca_V1.1$ currents, suggesting that STAC3 plays a role in determining the slow $Ca_V1.1$ currents kinetics. To determine if STAC3 does indeed slow down the CDI of $Ca_V1.1$ currents, we found that $Ca_V1.1$ displays negligible CDI, which is not affected by STAC3, and that STAC3 inhibits the VDI of $Ca_V1.1$ currents. To further investigate the effect of STAC proteins on VDI, we performed experiments on other LTCC. We found that STAC proteins inhibit the VDI of $Ca_V1.2$ and $Ca_V1.3$. Using the ETLAAA mutant, we could demonstrate that the inhibition of VDI relies on the same STAC linker region as CDI. Experiments co-expressing $Ca_V1.3$ with β2a with or without STAC3 revealed that STAC proteins inhibit VDI using a different mechanism compared to membrane tethered β subunits (β2a, β2e).
Discussion: Taken together, our results show that STAC3 slows down the kinetics of activation and inactivation of $Ca_V1.1$ and that STAC proteins inhibit not only the CDI, but also the VDI of LTCC currents.
Acknowledgements: This research was supported by the Austrian Science Fund FWF (P33776 and DOC30-B30).
Keywords: STAC proteins – STAC3 – calcium-dependent inhibition – voltage-dependent inhibition – calcium channels – $Ca_V1.1$ channels – L-type calcium channels

A2.3

The effect of bile acids on the expression of doxorubicin reductases

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Background: Aldo-keto reductase (AKR) and carbonyl reductase (CBR) are involved in NADPH-dependent two-electron reduction of the anticancer drug doxorubicin into the less active and cardiotoxic

metabolite doxorubicinol. As endogenous signalling molecules, bile acids have a propensity to modulate gene expression of numerous drug-metabolizing enzymes. The aim of this study was to assess the influence of the bile acids ursodeoxycholic acid (UDCA) and chenodeoxycholic acid (CDCA) on the expression of *AKR1A1* and *CBR1* in the MCF7 cell line.

Methods: Human breast adenocarcinoma MCF7 cells were treated with 0.25 μM doxorubicin (D) or co-treated with doxorubicin and either 50 μM of ursodeoxycholic acid (DU) or 6 μM of chenodeoxycholic acid (DC). Cells were harvested after 24 h, RNA was isolated and transcribed to cDNA. The expression of *AKRA1* and *CBR1* mRNA was assessed using RT-qPCR compared to *ACTB* (β-actin) as a reference gene. Gene expression was analysed using the comparative $2^{-\Delta\Delta C_T}$ method, and data were analysed by one-way ANOVA and Tukey's *post hoc* test.

Results: The expression of *AKR1A1* in group D was increased 1.1-fold compared to untreated cells. Expression of *AKR1A1* was upregulated in group DU (11.49 ± 1.83-fold *vs*. control: $p < 0.001$; 10.65 ± 1.69-fold *vs*. D, $p < 0.001$), and in group DC (27.91 ± 3.58-fold *vs*. control, $p < 0.001$; 25.86 ± 3.31-fold *vs*. D, $p < 0.001$). *CBR1* expression in group D was increased 2.78 ± 0.59-fold *vs*. control ($p = 0.946$). Co-treatment with ursodeoxycholic acid reduced *CBR1* expression compared to group D (−1.57 ± 0.34, $p = 0.560$), whereas *CBR1* was upregulated in group DC (14.38 ± 5.72 *vs*. control, $p < 0.001$; and 5.23 ± 2.08 *vs*. D, $p < 0.001$).

Discussion: The reduction of doxorubicin catalysed by the AKR and CBR family member enzymes render the formation of the C13 alcohol metabolite doxorubicinol, which has reduced antineoplastic activity and higher cardiotoxic potential than the parent compound. By upregulating the expression of *AKR1A1* and *CBR1*, chenodeoxycholic acid and ursodeoxycholic acid may affect the therapeutic efficacy and the risk of doxorubicin-related heart failure.

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Keywords: bile acids – doxorubicin – cardiotoxicity

A2.4

The effect of selected substances on the modulation of energy metabolism in human endothelium

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Background: Human endothelial cells are the first cells that come in direct contact with a drug after intravenous application. These cells are usually in a quiescent state in which they fill their energy demands through glycolysis. Upon the presence of stress or pathologic changes, they can quickly adapt and differentiate into proliferative cells. This increases energy demand, which pushes the cells to increase energy production through oxidative phosphorylation. Mitochondrial respiration leads to oxidative stress, which is the principal risk factor for the development of endothelial dysfunction. Thus, the modulation of energy metabolism has great potential in preventing cardiovascular disease.

Methods: Here, we studied the effect of selected drug substances on the energy metabolism in the human endothelial cell line EA.hy926 under physiological (pH 7.4) and acidic (pH 7.2) conditions. The extracellular flux analyzer Agilent Seahorse XFe24 enabled us to observe the extracellular acidification rate (glycolysis) and the oxygen consumption rate (mitochondrial respiration) in real time. We used substances from four different pharmacological groups: non-opioid analgesics (paracetamol, ibuprofen, acetylsalicylic acid), antipsychotics (haloperidol, fluphenazine, risperidone), antidepressants (citalopram, amitriptyline, fluoxetine) and substances with vascular effects (adrenaline, N^{ω}-nitro-L-arginine, acetylcholine, sildenafil). Following a literature review, we selected concentrations in the range corresponding to upper therapeutic or low-toxic plasma levels. In parallel, we tested the cell viability under the same experimental conditions with a resazurin reduction assay.

Results: Our results showed that paracetamol altered oxidative phosphorylation in the endothelium. Ibuprofen demonstrated a damaging effect on mitochondria, presumably due to uncoupling electrochemical potential on the inner mitochondrial membrane leading to increased production of reactive oxygen species. Also, acetylsalicylic acid was mitotoxic to endothelial cells in acidic conditions. Interestingly, our experiments showed a correlation between nitric oxygen (NO) levels and glycolysis. Inhibition of endothelial nitric oxide synthase (eNOS) with N^{ω}-nitro-L-arginine increased the rate of glycolysis. Decreased levels of NO are a common feature of endothelial dysfunction, thereby suggesting that glycolysis enzymatic systems are potential new therapeutic targets. Other studied drug substances did not affect the energy metabolism of endothelial cells; thus, they did not exhibit any mitochondrial toxicity.

Discussion: Our results showed that some of the studied drug substances, which are not cytotoxic on routine tests of cell viability, can act mitotoxic with unfavorable effects on the energy phenotype of endothelial cells.

Acknowledgements: This work was financially supported by the Slovenian Research Agency (research program P3-0067).

Keywords: endothelium – energy metabolism – glycolysis – mitochondria – oxidative phosphorylation

A2.5

Effect of selected drug compounds on dopamine cytotoxicity on human endothelial cells and rat astrocytes

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Background: Increased dopamine levels damage neurons and other cells in the central nervous system, thus leading to the accelerated progression of neurodegenerative diseases. The presumed mechanism of dopamine neurotoxicity is its enzymatic metabolism via monoamine oxidase B (MAO-B) to reactive metabolites with corresponding oxidative stress. Our research aimed to examine the influence of dopamine on endothelial cells and astrocytes, as well as the potential protective effects of selected drug compounds to ameliorate dopamine toxicity.

Methods: Human endothelial cells and isolated astrocytes from the cortex of neonatal rats were prepared to study the effect of dopamine on their viability depending on the dopamine concentration, exposure time, and the presence of selected drugs. To study the role of dopamine metabolism, all cells were pre-incubated with inhibitors of MAO-B and catechol-*O*-methyltransferase (COMT). To assess the role of dopamine cellular uptake, we used inhibitors of the serotonin transporter (SERT) and the noradrenaline transporter (NET). To study the role of oxidative stress, we used the antioxidant quercetin. We have also measured oxidative stress using the CAA method. To

study the impact of dopamine and selected drugs on mitochondrial function, we used the Seahorse Cell Mito Stress Test kit from Agilent.
Results: Dopamine-induced time- (2–48 hours) and concentration-dependent (1 nM–1 mM) cytotoxicity was assessed on both cell lines. Inhibition of dopamine metabolism by several MAO-B and COMT inhibitors did not increase cell viability. Similarly, inhibition of dopamine uptake by SERT and NET inhibitors had no effect. However, the reduction of oxidative stress by quercetin increased cell viability. We confirmed by the CAA method that dopamine increased cellular oxidative stress and that neither MAO-B, COMT, SERT and NET inhibitors ameliorated this condition. However, quercetin decreased oxidative stress in both cell lines. Likewise, the Mito Stress Test assay showed altered mitochondrial function due to dopamine, and no mitochondrial protection offered by inhibiting its metabolism or cellular uptake.
Discussion: Our results show that dopamine cytotoxicity is concentration- and time-dependent. Inhibition of MAO-B and COMT did not change the cell viability. This observation suggests that the mechanism of cell damage does not originate predominantly from dopamine metabolism but rather from its non-enzymatic auto-oxidation. We speculate that dopamine autoxidation products lead to increased oxidative stress that was responsible for observed cellular and mitochondrial toxicity. Indeed, the protective effect of quercetin confirmed this hypothesis.
Acknowledgements: This work was financially supported by the Slovenian Research Agency (research program P3-0067: Pharmacology and Pharmacogenomics).
Keywords: cytotoxicity – dopamine toxicity – mitochondrial function – oxidative stress

A2.6

Antihyperlipidemic potential of a carob extract (*Ceratonia siliqua* L.) in high-fat diet-fed rats

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Background: The carob tree (*Ceratonia siliqua* L.) is widely spread in the Mediterranean region but in production and use it becomes popular throughout the world. In recent years, carob has been in the focus of many studies related to the use of carob products in animal and human nutrition. Furthermore, carob pulp has been recognized as a valuable source of polyphenols. The objective of the present study was to investigate the hypolipidemic effects of carob extract on lipid status, and liver and kidney function in rats exposed to cholesterol-fortified food.
Methods: The study was conducted in male adult Wistar rats, divided into control and experimental groups. Rats weighed 200–230 grams, but their body weight increased rapidly due to the high caloric value of their food. Standard pellet food for laboratory rats was enriched with 3% of cholesterol and 0.5% of cholic acid. Microwave-assisted extraction was performed and the carob extract obtained at optimal conditions was used in the study. The animals were brought from the Military Medical Academy (Belgrade, Serbia). During the experiment, the animals were kept in standard laboratory conditions. Animal care and all experimental procedures were carried out in accordance with EU Directive 2010/63/EU on the protection of animals used for scientific purposes and the Law of Animal Welfare of the Republic of Serbia (OG RS 41/09). The study was conducted after obtaining the approval of the Ethics Commission for the Protection of Laboratory Animal Welfare. The study lasted for 4 weeks and at the end of the experiment all animals were anesthetized with urethane and sacrificed by cardiac punction after which blood samples were collected for further analysis.
Results: In order to investigate the influence of the carob extract on liver function, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and total and direct bilirubin were measured. The results showed that activities of transaminases in animals treated with carob extract were lower in comparison to control, while concentrations of direct and total bilirubin showed no difference between groups. Nephroprotective properties of the carob extract were also investigated. Concentrations of urea, creatinine and uric acid showed no statistical significance between the groups. The lipid profile was determined in serum using commercially available kits based on well-established spectrophotometric methods. Triglycerides and total, LDL and HDL cholesterol levels were significantly lover in groups treated with carob extract and simvastatin compared to control.
Discussion: In this research, we determined the influence of a carob extract on lipid status, indicators of hepatic and renal function in high-fat diet-fed rats. Our study showed that carob extract has potential as an adjuvant in the treatment of dyslipidemia.
Acknowledgements: This research was supported by the Provincial Secretariat for Higher Education and Scientific Research of Vojvodina (project no. 142-451-2574/2021-01 and project no. 142-451-2331/2022-01).
Keywords: carob extract – lipid-lowering effects – phytotherapy

A2.7

Effects of selective 5-HT$_{2A}$ agonists and antagonists on cell metabolism and mitochondrial function in neonatal primary astrocytes

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Background: The 5-HT$_{2A}$ receptor is one of the most widely spread G protein-coupled receptors in the central nervous system. It is also expressed in a number of cells in peripheral tissues. It is involved in regulation of such diverse functions as smooth muscle contraction, platelet aggregation, arousal and mood. The effects of its activation have mostly been studied on neurons. Recently, its important role in other cells have been discovered. Importantly, serotonin has been shown to regulate mitochondrial biogenesis via 5-HT$_{2A}$ receptors. Our aim was to study similar effects on neonatal astrocytes, which express 5-HT$_{2A}$ receptors.
Methods: We examined the effect of the selective 5-HT$_{2A}$ agonist 2,5-dimethoxy-4-iodoamphetamine (DOI), serotonin (5-HT) as a nonselective agonist of serotonin receptors, and the selective 5-HT$_{2A}$ antagonist ketanserin on cell viability and cellular metabolism of primary neonatal astrocyte cell cultures. We administered different concentrations of 5-HT (0.1–10 µM), DOI (0.1–10 µM), ketanserin (5–50 µM), as well as combination of serotonin and DOI with ketanserin for 48 hours before determining the energy phenotype. To characterize the energy phenotype of astrocytes, we measured oxygen consumption rate and extracellular acidification rate using Agilent Seahorse. To assess the mitochondrial function in more detail, we calculated basal respiration, maximal respiration, proton leak, spare respiratory capacity and coupling efficiency.
Results: We observed no effect of serotonin and DOI (5-HT$_{2A}$ agonists) on cell viability in all studied concentrations, whereas ketanserin (a 5-HT$_{2A}$ antagonist) decreased cell viability at high

concentrations. We discovered that DOI increased basal respiration of neonatal astrocytes, while 5-HT had no effect.

Discussion: In light of recent discoveries of the importance of astrocytes in neuroprotection and their ability to transfer functional mitochondria [1] we hypothesize that some of the postulated neuroprotective effects of 5-HT_{2A} receptor agonists are a consequence of an increased mitochondrial biomass in supportive tissue astrocytes. Our results show a change in cellular metabolism of astrocytes when selective 5-HT_{2A} receptor ligands are applied. Indeed, the oxygen consumption rate increased at all studied concentrations of selective 5-HT_{2A} agonists. In conclusion, further studies are warranted to confirm that observed changes in astrocyte energy phenotypes are specific 5-HT_{2A}-receptor-mediated effects.

Acknowledgements: This work was financially supported by the Slovenian Research Agency (research program P3-0067: Pharmacology and Pharmacogenomics).

Keywords: 5-HT_{2A} receptors – astrocytes – energy metabolism – mitochondria – serotonin

Reference

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A2.8

Relevance of hydrogen bonds in binding and functional properties of histamine H_2 receptors expressed in rat astrocytes

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Background: The histamine H_2 receptor whose binding domain exists in a small pocket within a lipid bilayer is a typical representative of G protein-coupled receptors. A crucial contribution in the binding of histamine to the H_2 receptor is the formation of three hydrogen bonds between amino-acid residues within the third and the fifth transmembrane α-helices (Asp^{98}, Asp^{186} and Tyr^{250}) and three nitrogen atoms of the histamine molecule.

Methods: In order to estimate the relevance of hydrogen bonds in the process of binding of ligands to the H_2 receptor and further on its function, we compared the binding properties of histamine to H_2 receptor binding sites on cultured neonatal rat astrocytes and isolated membranes from cultured astrocytes in control and deuterated medium. Further on, we determined cAMP production upon stimulation with agonist in control and deuterated conditions.

Results: The specific [^{3}H]tiotidine binding was inhibited by histaminergic agonists and antagonists. Deuteration significantly increased the affinitiy of histamine to displace specific [^{3}H]tiotidine binding in whole astrocyte cells, since pK_i changed from 7.5 ± 0.11 (control) to 8.1 ± 0.16 (D20 medium; $p < 0.05$). However, in isolated rat astrocyte membranes, pK_i changed from 6.6 ± 0.03 (control) to 6.8 ± 0.1 (D20 medium). Deuteration affected the signalling properties of glial H_2 receptors, but only when a very low concentration of histamine was used.

Discussion: Replacing hydrogen atoms, which are involved in binding of histamine to the H_2 receptor, with deuterium atoms results in different lengths of intermolecular and intramolecular distances. This leads to a structural change of ligand and receptor binding sites which affects the binding affinities and functional characteristics. The ligand H/D substitution is relevant for therapy in the context of (per)deuterated and thus more stable drugs that are expected to enter the therapeutic practice in the near future. Moreover, the presented approach may contribute towards understanding receptor activation, while a distant goal remains the *in silico* discrimination between agonists and antagonists based on the receptor structure.

Acknowledgements: The work was supported by grants J1-2014, P3-067 and P1-012 of the Slovenian Research Agency.

Keywords: deuteration – histamine H_2 receptors – hydrogen bonds

A2.9

The uptake of [^{3}H]dopamine into cultured neonatal rat astrocytes involves multiple transporters

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Background: Dopamine transmission includes dopamine synthesis, release of dopamine from axonal boutons or dendrites, dopamine receptor activation, dopamine reuptake into the presynaptic neuron, enzymatic degradation or vesicular transport. Transporter-mediated uptake determines the duration and physical spread of released dopamine. Uptake of dopamine is mediated by uptake-1 transporters, such as dopamine and norepinephrine transporter (DAT and NET) characterized by dependence on Na^+/Cl^- ions with low capacity and high affinity, and Na^+/Cl^--independent uptake-2 transporters with lower affinity and higher capacity such as organic cation transporters (OCTs) and plasma membrane monoamine transporter (PMAT). The role of astrocytes, which ensheath the synapse in the dopamine homeostasis, has never been investigated in detail; however, it has been established that astrocytes mainly express enzymes for dopamine metabolism and receptors for neurotransmitters, and have been shown to respond to neuronal activity by changes in intracellular concentration of Ca^{2+}.

Methods: Astrocytes were cultured by a well-established protocol in our laboratory. Neonatal rat cortices and striata were excised and prepared to produce primary astrocytic cells that were grown on 12-well plates for three weeks. Kinetic uptake studies were performed by 20-minute incubation of various concentrations of [^{3}H]dopamine at 37 °C and 4 °C to determine the specific and non-specific uptake of dopamine. Inhibitory studies included the use of the antidepressants desipramine, nortriptyline and amitriptyline, as well as the DAT-specific inhibitor GBR 12909 and the substances decynium-22 and corticosterone. Total RNA was extracted from astrocytic cells as well as from cortical, striatal and kidney neonatal rat tissue. The quantity of mRNA of the transporters DAT, NET, OCT1, OCT2, OCT3 and PMAT was determined by quantitative polymerase chain reaction (qPCR). Actin was used as an endogenous control. Protein expression of DAT, NET and PMAT was determined by western blot. Neonatal rat tissue samples (cortex, striatum and kidney) were used as positive controls.

Results: Dopamine uptake into cultured striatal and cortical neonatal rat astrocytes is time-, temperature- and concentration-dependent. Saturation and inhibition curves of dopamine uptake show characteristics of transport involving multiple carriers. Further on we found mRNA for NET, PMAT, OCT1, OCT2 and OCT3 in cortical and striatal astrocytes. mRNA expression of NET and PMAT appears to be most abundant in both types of glial cells. Western blot confirmed the presence of NET only. mRNA as well as protein expression of DAT appears to be very slight.

Discussion: Astrocytes perfom a variety of homeostatic functions; however, their role in the dopamine synapse has yet to be established. Dopamine is taken up into cultured striatal and cortical rat

astrocytes by both uptake-1 and uptake-2 transporters. NET appears to have a greater role in astrocytic dopamine uptake than DAT.

Keywords: astrocytes – dopamine uptake – monoamine transporters – dopamine transporter – norepinephrine transporter

A2.10

A new role of the N-terminus in folding and intracellular trafficking of the human creatine transporter 1

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Background: The human creatine transporter 1 (CRT1, SLC6A8) is a member of the sodium-dependent neurotransmitter transporter (NTT) protein family. Creatine transporter deficiency (CTD) has been associated with a number of disorders, ranging from epilepsy to mental retardation, autism, development delay, behavior problems, motor dysfunction or gastrointestinal symptoms. Folding and trafficking defects in SLC6 proteins frequently lead to pathological conditions; *e.g.* mutations in the human creatine, dopamine and GABA transporters trigger CTD, parkinsonism and epilepsy, respectively. Treatment with the chemical chaperone 4-phenylbutyrate (4-PBA) rescues the surface expression and uptake activity of several folding-deficient CTD variants. Hence, it is vital to decipher the arcane molecular machinery behind the protein folding and intracellular trafficking of hCRT1.

Methods: The following methods were used: (i) mutagenesis, to create serial truncation mutants along the N-tail, as well as single/double point mutations in other regions of hCRT1 (QuikChange kit); (ii) biochemical (western blotting and immunoprecipitation) and pharmacological characterization (specific [^{3}H]creatine uptake assays) of wildtype and mutant transporters in transiently transfected HEK 293 cells; (iii) immunocytochemistry analysis of C- and N-tail yellow fluorescent protein (YFP)-tagged hCRT1 and several serial truncation mutants' localization at the cell surface and ER, respectively.

Results: Using biochemical and pharmacological approaches, we observed that CRT1 is the only NTT intolerant to introducing a YFP tag at its N-terminus, *i.e.* resulting in ER retention. We generated serial truncations along the N-tail of CRT1 and found that the truncated mutant of ΔN51-CRT1 abolished creatine uptake. In addition, N-tail YFP-tagged hCRT1 and ΔN60-CRT1 mutants are located in ER in transiently transfected HEK 293 cells.

Discussion: Our findings break a hallmark rule, previously established for SLC6 transporter relatives of hCRT1, that their amino tails are virtually dispensable to their folding and trafficking (cell surface expression) or even their substrate uptake activity. Our data provide novel insights into the molecular and physiological features underlying the non-conforming folding and trafficking routes of CRT1. These ought to impart crucial details relevant to the role and regulation of CRT1 in disease (*e.g.* CTD or cancer).

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Keywords: creatine transporter 1 – creatine transporter deficiency – protein folding – protein trafficking

A2.11

Two-pore channels are crucial regulators of mast-cell activity and anaphylaxis

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Background: Allergic and anaphylactic reactions are aberrant immune responses to exogenous, naturally harmless substances. The worldwide prevalence of allergies is continuously increasing over the last decades. Mast cells and basophils are essential drivers of these diseases, releasing inflammatory mediators such as histamine.

Methods: Utilizing knockout mouse models, we investigated the role of endo-lysosomal two-pore channels in the function of mast cells and anaphylactic reactions *in vivo* and *ex vivo*. Using electrophysiologic, fluorometric imaging and molecular biologic approaches, we studied TPC1-dependent signaling in mast cells.

Results: We were able to link the endo-lysosomal two-pore channel TPC1 to systemic anaphylaxis *in vivo* and underlying mast-cell function *ex vivo*. *Tpc1*-deficient mice develop enhanced systemic anaphylaxis reflected by a drop in body temperature and slower recovery compared to wild-type animals. Genetic deletion or pharmacologic inhibition of TPC1 enhances mast-cell degranulation and histamine release. *Tpc1*-deficient mast cells displayed augmented calcium signals, originating from the endoplasmic reticulum (ER) calcium stores in response to IP_3 stimulation. We hypothesize that TPC1 plays an essential role in the regulation of the inter-organellar calcium homeostasis in mast cells, indirectly controlling endo-lysosomal calcium uptake and filling of ER calcium stores and thus exocytosis in mast cells [1]. However, the structural and molecular basis of such endo-lysosomal–ER interactions remains to be clarified. Our results indicate massive contact areas between these organelles, which crucially depend on TPC1 activity [2].

Discussion: Accordingly, it is tempting to speculate that activation of TPC1 ameliorates mast-cell degranulation, highlighting TPC1 as a potential drug target against allergic hypersensitivity.

Keywords: allergy – anaphylaxis – calcium homeostasis – mast cells – organellar ion channels – TPC1 – two-pore channels

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A2.12

A novel scaffold opioid ligand as a κ-opioid receptor antagonist: a pharmacological and computational study

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Background: The κ-opioid receptor (KOR), a G protein-coupled receptor (GPCR) and a prominent member of the opioid receptor family, has gained increased consideration to drug discovery over the recent years. Targeting the KOR is regarded as a promising strategy for the treatment of numerous human disorders where the κ opioid system plays a central role, with special attention directed to KOR antagonists as innovative therapies for CNS disorders. A new scaffold opioid ligand (compound A: 4-[(2,3-dichlorophenyl)methyl-amino]-2-methylquinoline-8-carboxamide) was originally found as a μ-opioid receptor (MOR) antagonist, but its binding/selectivity and activation profile at the KOR and DOR (δ-opioid receptor) have not been investigated. In this study, we report on the *in vitro*, *in vivo* and *in silico* characterization of compound A by revealing this ligand as a KOR antagonist.

Methods: *In vitro* radioligand competitive binding and [^{35}S]GTPγS functional assays were performed with membranes from Chinese hamster ovary (CHO) cells stably expressing the human KOR or DOR (CHO-hKOR and CHO-hDOR cells, respectively). *In vivo* KOR antagonism was assessed in two pain models (acetic acid-induced writhing assay and the formalin test) in mice after subcutaneous (s.c.) administration. For molecular docking, Corina v3.00 was used to generate the 3D conformation of compound A and docking was performed using the GOLD (version 5.2). Molecular dynamics (MD) simulations were carried out using Maestro v2020-4 for system setup, OPLS 2005 force field for system parametrization and Desmond v2020-4 for performance of the simulations. Dynamic pharmacophores were generated with the Dynophore software (version 0.1). Percepta software was used to calculate the physicochemical properties (partition and distribution coefficients, *c*LogP and *c*LogD$_{7.4}$, respectively).

Results: In radioligand competitive binding assays, compound A bound at the KOR, albeit with moderate affinity (in low micromolar range), but with increased affinity than to the MOR, and without specific binding at the DOR, thus displaying a preferential KOR selectivity profile. Behavioural investigations in mice established the *in vivo* KOR antagonist properties of compound A after s.c. administration. Compound A effectively reversed the antinociceptive effects of the prototypical KOR agonist, U50,488, in the writhing assay and the formalin test. *In silico* investigations established structural determinants responsible for opioid receptor subtype selectivity of compound A. MD simulations and dynamic pharmacophore (dynophore) generation revealed differences in the stabilization of the chlorophenyl moiety of compound A within the binding pocket rationalizing the experimentally determined affinity values. Furthermore, compound A shows favourable physicochemical features and a better capability to enter the CNS (based on the *c*LogP and *c*LogD$_{7.4}$) compared to the known KOR antagonists.

Discussion: This comprehensive study aided by experimental pharmacological (binding and functional *in vitro* assays and behavioral nociceptive models) and computational (*in silico* methods) approaches established compound A as a novel KOR antagonist, with a structurally distinct scaffold compared to the so far known KOR ligands. This new chemotype represents a valuable starting point for chemical optimization toward the development of potential KOR therapeutics.

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Keywords: κ-opioid receptor antagonists – receptor binding studies – *in vivo* antagonism – molecular docking – molecular dynamics simulations

A2.13

Impact of pH on activity of the novel cephalosporine cefiderocol in pooled human urine

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Background: Antibiotic activity of different antibiotics can be impacted by pH, enhancing or reducing their bactericidal properties. Cefiderocol, a novel cephalosporine antibiotic is indicated for the treatment of infections caused by gram-negative bacteria such as Enterobacteriaceae and *P. aeruginosa*. These pathogens are also associated with complicated urinary tract infections. To better link *in vitro* experiments to *in vivo* conditions, pooled human urine (iron levels ~0.05 mg/l/24 h) and cation-adjusted Mueller-Hinton broth (CAMHB), were used as media to test cefiderocol activity against the aforementioned pathogens at pH 5–8.

Methods: Minimal inhibitory concentration (MIC) determinations were done according to CLSI guideline with the broth microdilution method of 17 clinical isolates of *E. coli* and ATCC 25922 (including isolates with extended-spectrum β-lactamase activity (ESBL)), 17 clinical isolates of *K. pneumoniae* and ATCC 700603 (also with ESBL), and 6 clinical isolates of *P. aeruginosa* and ATCC 27853. All MIC determinations (at least 3x up to 18x per strain) were conducted in pooled human urine and CAMHB at pH 5, 7 and 8. Human urine was obtained from young, male, healthy volunteers, frozen, thawed, pooled, sterile filtered (2 μm) and frozen again until usage. The pH was set directly before the start of the experiments with HCl or NaOH.

Results: MIC values in urine and CAMHB were overall identical and did only vary sporadically in a 1-fold dilution up or down. The median MIC values of both antibiotics where up to 50-fold higher in pH 5 than in pH 7 for *P. aeruginosa* isolates and 32-fold higher in *E. coli* and *K. pneumoniae* isolates, leading to MIC values above the EUCAST breakpoint (for iron-depleted CAMHB), for systemic infection of > 2 mg/l at an acidic pH (table 1).

Discussion: Acidic pH had a significant negative impact on the activity of cefiderocol in pooled human urine and may be explained by an altered availability of free ferric iron (required for optimal uptake into the cell). Moreover, since non-iron-depleted CAMHB was used, overall MICs might be elevated. Nevertheless, after a recommended intravenous administration of 2 g every 8 hours a concentration of 1247 mg/l of cefiderocol can be achieved in urine, suggesting that efficient killing of all tested pathogens could have been achieved even under acidic conditions *in vivo*.

Table 1: **MIC values of cefiderocol for all isolates and ATCC strains**

	MIC in CAMHB			MIC in urine		
	pH 5	pH 7	pH 8	pH 5	pH 7	pH 8
P. aeruginosa						
Median MIC of 6 isolates	8	0.15	0.14	7	0.14	0.08
ATCC 27853	8	0.25	0.25	8	0.25	0.125
E. coli						
Median MIC of 17 isolates	4	0.125	0.19	4	0.125	0.09
ATCC 25922	1	0.06	0.125	1.5	0.125	0.06
K. pneumoniae						
Median MIC of 17 isolates	16	0.5	0.31	16	0.5	0.31
ATCC 700603	8	0.25	0.125	8	0.5	0.25

Values are given as mg/l for pH 5, 7 and 8 in cation-adjusted MHB (CAMHB) and in urine.

Acknowledgements: Cefiderocol was thankfully provided by Shionogi Pharma Co.

Keywords: cefiderochol – antibiotic activity – pH – minimal inhibitory concentrations – time–kill curves

A2.14

The Drug Information Unit of the Medical Faculty of Novi Sad – Serving medical practitioners for 15 years

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Background: In Serbia, official drug-related data for medical professionals usually are provided by the National Agency of Drugs and Medical Devices or international databases. The Medical Faculty, University of Novi Sad, is hosting a Drug Information Unit which serves as regional drug information centre providing official information on drugs for the last 15 years to medical professionals in Vojvodina (northern region of Serbia, with a population of about 2,000,000 inhabitants). The aim of the study was to analyse requests on drug information sent by medical practitioners to the Drug Information Unit at the Medical Faculty, University of Novi Sad.

Methods: Data on requests on drug information sent to the Drug Information Unit in the last 15 years (starting from 2007), were collected and analysed. Data were analysed by two criteria: type of the client sending the request (general practitioners, specialist in medicine, nurses) and the type of information requested (*e.g.* drug interactions, side effects, use of drugs in pregnancy and lactation etc.).

Results: During the observed period of 15 years there were 2,720 requests on drug information generated by health-care professionals. The majority of requests were sent by specialists in different fields of medicine (73%), 24% by general practitioners and the rest (3%) by nurses. The most frequent type of information by health-care professionals were related to treatment of choice in bacterial infections (17.6%), drug–drug or drug–food interactions (16.3%) and safety of drug use during pregnancy and lactation (15%). Other inquiries were related to adverse drug reactions (14.5%), pharmacokinetics, most frequently related to drug pharmacokinetics in kidney and/or liver failure (13.7%), posology (9.6%), and treatment of choice in diseases where there is no consensus regarding treatment options (9.1%).

Discussion: The Drug Information Unit is a useful source of official information for all types of medical professionals offering various information on different topics related to drugs. In health-care professionals there is a growing demand for information on the use of antibacterial drugs, especially in case of drug-resistant bacteria, drug interactions most frequently caused by polypharmacy, and information on pharmacokinetics of drugs especially in patients with failure of excretory organs. Information provided by the Drug Information Unit improves the efficacy and safety of pharmacotherapy.

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Keywords: drug information – pharmacotherapy – drug efficacy – drug safety

A2.15

Targeting gut microbiota for the individualization of thiopurine therapy of inflammatory bowel disease

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Background: Accumulating evidence shows that gut microbiota (GM) are able to influence the efficacy and toxicity of certain drugs and that treatment outcomes vary greatly among individuals due to the variability of the GM. Generally, clinical trials do not include studies aimed to examine microbiota–drug interactions, leaving knowledge gaps on important pharmacokinetic properties of orally administered drugs. This study aimed to highlight the potential of GM as key players for the implementation of a personalized approach in the thiopurine therapy of inflammatory bowel disease (IBD).

Methods: The data on the thiopurine therapy–GM interactions have been provided from a review of original scientific articles published between 2000 and 2022. The search was performed using the following keywords: 'thiopurine therapy', 'gut microbiota', 'precision medicine', and 'microbial metabolism'.

Results: Thiopurines are the most commonly used drugs for the maintenance of remission of IBD. However, considerable interindividual variability in the clinical response, with approximately 40% of patients who are refractory to thiopurine therapy and 15–28% experiencing adverse events, is the main reason for switching to biologics. Considering that thiopurines have a very complex metabolic pathway, the GM, with their great metabolic power, might play an important role in interindividual variability. It has already been demonstrated that *Escherichia coli*, *Enterococcus faecalis* and *Bacteroides thetaiotaomicron* are equipped with the enzymes capable of targeting the metabolic pathway of thiopurines [1]. Additionally, a recent study has confirmed that the accumulation of

drugs by GM, even without biotransformation, largely affects outcomes for the majority of the studied drugs [2].

Discussion: Optimization of thiopurine therapy using a personalized treatment approach is clearly desirable before discontinuation of these drugs. Pre-treatment testing of enzymes involved in thiopurine metabolism, such as thiopurine methyltransferase (TPMT) is recommended for the therapeutic management of thiopurines by most international guidelines. Also, thiopurine treatment is monitored routinely in many laboratories by measuring metabolite concentrations in erythrocytes using high-performance liquid chromatography (HPLC) methods. Nevertheless, the drug treatment outcomes remain largely unpredictable. Therefore, further analysis of the interactions between thiopurines and GM is an important aspect which may lead to identifying novel tools for the prediction of response to the treatment and implementation of personalized IBD therapy based on GM.

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Keywords: drug metabolism – gut microbiome – precision medicine – thiopurines

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A2.16

In-home drug inventory among students – storage, use and disposal of drugs

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Background: Direct inspection of medicines stored in households allows insights into medicine use, storage, and disposal. Habits of students regarding medicines differ from the general population due to sociodemographic and other specific differences. This study aimed at describing patterns of medicines stored at students' home pharmacies, determine the volume of prescribed medicines and the rate of self-medication, as well as the medication storage and disposal habits among students in Novi Sad.

Methods: This cross-sectional study was performed in 70 student accommodations in Novi Sad in the period from 1 November 2018 until 20 December 2018. The study consisted of visiting student dormitories and making a direct insight into inventory of medicines based on the ATC classification and a semi-structured interview.

Results: All surveyed students' accommodations stored medicines. A total of 337 packages was found with the majority (71.4%) kept in one designated place, a home pharmacy. Drugs that affect the nervous system, the muscle–bone system and anti-infectives were the most common medicines in home pharmacies. Specifically, the drugs found in largest quantities were ibuprofen, paracetamol and diclofenac, accounting for 30.9% of the total number of drugs. Over 70% of medicines were purchased for self-medication. Antibiotics accounted for 5.9% of total drugs found, and from 20 packages of antibiotics, 6 were obtained without prescription (30%). About 10% of students stored expired drugs, 75% of medicines were kept properly, but a negligible part of them was properly disposed. Even though the majority of students (74.3%) considered that throwing medicines into the garbage and toilet is bad for the environment, most (41.4%) answered that this type of disposal is the easiest and the most convenient method of drug disposal. The majority of drugs in solid or semisolid pharmaceutical forms were disposed together with the household garbage (67.1%), and the same was shown for liquid forms (62.9%). Less than 3% of the respondents stated returning drugs to the pharmacy to be properly disposed.

Discussion: Several specific differences with respect to the general population in Novi Sad were determined: a lower rate of prescribed medicines and medicines used for chronic illnesses, but a higher rate of self-medication in the student population. Habits regarding antibiotics showed similar patterns as observed previously. About half of the antibiotics found were not currently in use, supporting the finding that antibiotics stored at home are an important source of drugs used for self-medication. Antibiotic leftovers also raise an issue of medicine non-adherence. Habits regarding drug storage and disposal were similar to the results obtained in our previous studies examining home pharmacies of the general population. Self-medication is very common among students. Although the majority of medicines are stored properly, they are disposed of in an environmentally unfriendly manner.

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Keywords: drug utilization – home pharmacies – self-medication – pharmacoepidemiology

A2.17

Knowledge on arterial hypertension therapy in outpatients in Novi Sad

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Background: Factors such as obesity, smoking, poor eating habits and sedentary life-style, and high levels of cholesterol all can contribute to higher rates of cardiovascular diseases. Despite the availability of a large number of medicines efficient in hypertension

management, the rate of successfully controlled hypertension in Serbia was found to be low. Previous studies in our setting found prescription patterns in line with available guidelines, but information on patient-related factors possibly influencing blood-pressure control is lacking. Involving patients in decision-making regrading treatment, and providing information about the life-style habits that can affect blood-pressure control should be a routine part of care. Therefore, this study aimed to determine levels of knowledge on risk factors, symptoms, treatment and complications of hypertension in out-patients currently being treated with antihypertensive drugs.

Methods: This research was conducted on a random sample of 100 outpatients between 17 December 2018 and 31 January 2019 during dispensing of antihypertensive drugs in pharmacies. Patients completed a questionnaire containing four parts: demographic information, health status, knowledge on therapy for arterial hypertension and current practice of management of hypertension.

Results: Rates of uncontrolled hypertension, during the last check-up, were statistically significantly higher in men (52.3%), patients older than 65 years of age (44.0%) and pensioners (51.9%) compared to reference groups. A high level of knowledge of non-pharmacological measures of prevention of hypertension was observed, but misconceptions on hypertension symptoms were prevalent. Patients were well aware on the complications of hypertension. The highest percentage of examinees stated that they were using 2 medicines in therapy (46.8%), and that they were aware of the voluntary risks of therapy termination. About 30% of patients stated that they sometimes skip taking the prescribed therapy, listing forgetfulness as the most common reason.

Discussion: Adequate blood pressure control was achieved only in less than half of outpatients on antihypertensive therapy. The fact that included patients were the ones picking up their treatment in pharmacies could mean that the overall rates of blood-pressure control in patients prescribed antihypertensive drugs in the Serbian setting are even lower, as the patients with primary non-adherence and patients with non-persistence could not be included. Previous findings of male sex being associated with lower rates of blood-pressure control was confirmed in the present study. Results on the influence of age on blood-pressure control in the available literature are contradicting, but the observed lower rates of blood-pressure control in patients older than 65 years could possibly be explained by comorbidities, drug-taking habits and fear of interactions with other drugs. The biggest misconception identified in the present study was uncontrolled hypertension being associated with specific symptoms, while knowledge on other hypertension-related issues was adequate. Multiple other factors that could influence blood-pressure control could not be determined due to the nature of study design. Further reasearch is necessary to determine the culprits behind low rates of blood-pressure control in the Serbian setting.

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Keywords: arterial hypertension – pharmacological knowledge – pharmacotherapy

A2.18

Bacterial resistance and consumption of antibiotics in Serbia compared to neighbouring countries of the European Union for the period of 2014–2018

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Background: There has been suggested a positive relationship between the consumption of antibiotics and the development of resistance to them. Due to the increase in the overall consumption of antibiotics, bacteria have been put under pressure to survive and they have adapted by developing different resistance mechanisms. Therefore, in this research, the consumption of antibiotics and resistance to them was compared between Serbia and its neighbouring European Union countries, in order to notice possible differences or similarities.

Methods: This was a retrospective, observational study of routinely collected data for consumption of antibiotics and national rates of antimicrobial resistance in Serbia, Croatia, Hungary, Romania and Bulgaria in the period of 2014–2018. Data on antibiotic consumption in Bulgaria, Croatia, Romania and Hungary were taken from the website of the European Surveillance of Antimicrobial Consumption Network (ESACNet), while data on the resistance of microorganisms to the main groups of antibiotics were obtained from the Surveillance Atlas of Infectious Diseases. Data on the consumption of antibiotics in Serbia were obtained from the annual reports of the Agency for Medicines and Medical Devices of the Republic of Serbia (ALIMS). The source of data on the resistance of microorganisms to antibiotics was the Central Asian and European Antimicrobial Resistance Surveillance Network (CAESAR).

Results: After processing the collected data, Romania was presented as the country with the highest consumption of antibiotics in 2018 with 25 DDD/1000/day (DDD – defined daily dose), followed by Serbia (24 DDD/1000/day) and Bulgaria (21 DDD/1000/day). In contrast to the mentioned countries, Croatia (18.8 DDD/1000/day) and Hungary (14.8 DDD/1000/day) had a much lower consumption of antibiotics. Additionally, in the observed period from 2014 to 2018, the strongest trend of reducing the consumption of antibiotics was recorded in Croatia. When processing data on the consumption of the main groups of antibiotics in 2018, it was observed that β-lactams were the most consumed group of antibiotics in all five countries. Finally, the study confirmed the obvious correlation between antibiotic consumption and resistance to certain groups of antibiotics by several strains: three gram-negative (*Escherichia coli*, *Acinetobacter* spp., *Pseudomonas aeruginosa*) and two gram-positive microorganisms (*Staphylococcus aureus*, *Streptococcus pneumoniae*).

Discussion: Although there has been a change in the pattern of use and a reduction in the consumption of antibiotics in Serbia compared to previous years, the current results show that broad-spectrum antibiotics remain the first choice for the treatment of infections which was the case for the EU countries as well. Moreover, it was shown that overuse of antibiotics definitely leads to an increase in the resistance of some bacterial strains to them. For example, resistance of *Acinetobacter* spp. is extremely high for the main groups of antibiotics in all countries of our study. Overall, this study emphasizes the importance of the relation between antibiotic consumption and development of antimicrobial resistance.

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