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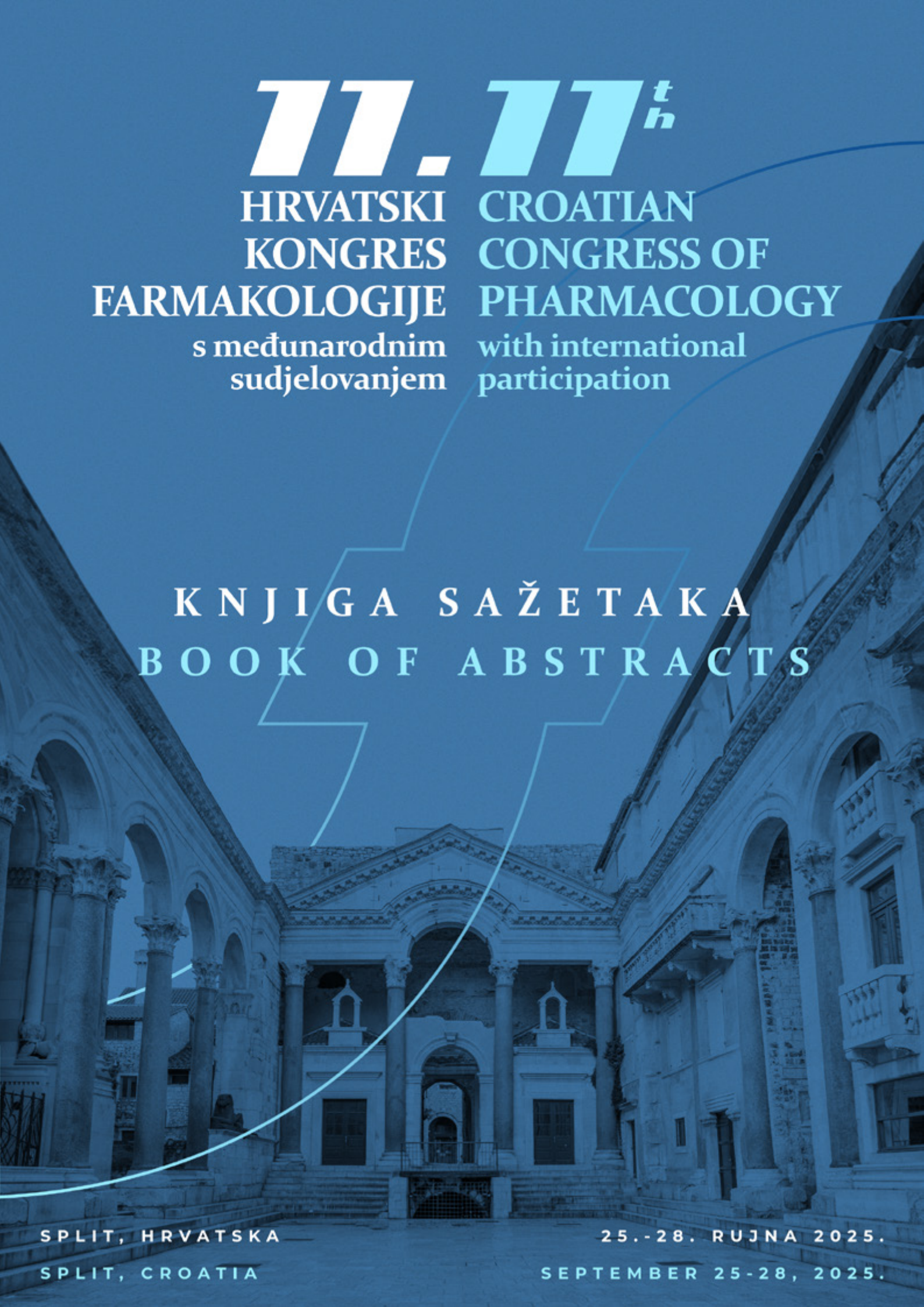

KNJIGA SAŽETAKA

BOOK OF ABSTRACTS

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PLENARNA PREDAVANJA / PLENARY LECTURES

PHARMACOGENOMICS - PAST, PRESENT, IS THERE A FUTURE?

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Pharmacogenomics (Pgx) is the study of how individual's genetic makeup affects response to medications in terms of both, efficacy and safety. Research in the field helped us to understand interindividual differences in drug response in different populations, and provided evidence needed for implementation of Pgx into clinical practice. Approximately 90% of individuals have an actionable genotype for at least one pharmacogene. However, adoption of Pgx into national health services across different countries has been inconsistent, and despite huge efforts to harmonise clinical guidelines, there are still some translational gaps. In my talk, I will briefly look into the history of pharmacogenetics, investigate the current state of research in this area, and envisage the progress in the field in the future.

PHARMACOTHERAPY OF COGNITIVE DISORDERS IN THE GERIATRIC POPULATION

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Dementias are complex neurodegenerative and cerebrovascular disorders that require distinct pharmacological strategies due to their diverse pathological mechanisms. Among the most prevalent forms are Alzheimer's disease, dementia with Lewy bodies, frontotemporal dementia, and vascular dementia. While no curative treatment currently exists to halt disease progression, recent advancements in Alzheimer's research have introduced therapeutic approaches aimed at slowing neurodegeneration. A dominant framework in Alzheimer's disease pathophysiology is the amyloid cascade hypothesis, which posits that the accumulation of β-amyloid (Aβ) in the brain initiates a sequence of neurodegenerative events, ultimately leading to widespread neuronal loss and cognitive impairment. Aβ is a ubiquitous protein with several essential functions, including synaptic regulation, injury recovery, microangiogenesis, inhibition of cell growth, antimicrobial activity, and maintenance of the blood–brain barrier. In individuals with Alzheimer's disease, Aβ accumulation begins 15 to 20 years before the onset of clinical symptoms. The etiology of this accumulation differs between early- and late-onset Alzheimer's disease: in early-onset cases, overproduction of Aβ drives pathological aggregation, whereas in late-onset cases, impaired clearance mechanisms are primarily responsible. Current symptomatic therapies do not facilitate Aβ clearance; however, experimental and clinical studies with monoclonal antibodies have demonstrated promising efficacy in reducing amyloid burden and slowing disease progression. Recently FDA-approved monoclonal antibodies, such as lecanemab and donanemab, have garnered significant attention for their ability to target Aβ fibrils, remove amyloid plaques, and mitigate cognitive decline. Clinical trials indicate that these therapies can slow cognitive decline by approximately 30 percent over 18 months. Lecanemab preferentially binds to Aβ protofibrils, preventing the formation of mature plaques, whereas donanemab binds to existing plaques, facilitating their rapid clearance via immune-mediated mechanisms. Both therapies, however, are associated with amyloid-related imaging abnormalities (ARIA), including cerebral edema and microhemorrhages. Notably, the incidence of ARIA is lower with lecanemab than with donanemab. To address the limitations of first-generation monoclonal antibodies, trontinemab, a novel therapeutic currently in late-stage development, employs a brain shuttle mechanism that enhances blood–brain barrier penetration. Unlike lecanemab and donanemab, trontinemab incorporates a transferrin receptor-binding domain, allowing superior brain penetration at lower doses, thereby improving efficacy while reducing systemic exposure and side effects. Despite their therapeutic promise, monoclonal antibodies require strict patient selection. These treatments are indicated exclusively for early-stage Alzheimer's disease, specifically in individuals with mild cognitive impairment or mild dementia, representing only 10 to 20 percent of patients. They provide no significant benefit in moderate or severe Alzheimer's disease, where extensive neurodegeneration has already occurred. Before treatment initiation, confirmation of Aβ pathology via PET imaging or cerebrospinal fluid biomarkers is mandatory. Additionally, APOE genotyping is strongly recommended due to its major influence on treatment safety. Carriers of the APOE ε4 allele, particularly homozygous ε4/ε4 individuals, have the highest risk of ARIA, with approximately 30–40 percent developing complications. Heterozygous ε3/ε4 carriers face a moderate risk, while non-carriers (ε3/ε3 or ε2/ε3) are at lower risk. Given these risks, APOE ε4 carriers require enhanced MRI surveillance throughout treatment. Comorbidities also affect the efficacy and safety of monoclonal antibody therapy. Patients with a history of stroke, cerebral microbleeds, or severe cardiovascular disease face an increased ARIA risk and may not be suitable candidates.

Similarly, patients on anticoagulant therapy, such as warfarin or direct oral anticoagulants, are at elevated risk of intracranial hemorrhage. Severe hepatic or renal impairment may also alter drug metabolism and clearance, necessitating careful assessment before treatment. Beyond clinical considerations, logistical challenges limit widespread use. Administration of monoclonal antibodies requires specialized infusion centers, trained medical personnel, and frequent MRI monitoring, which significantly increases healthcare burden. MRI scans are required at baseline and at regular intervals—such as one, three, and six months post-initiation—to monitor for ARIA. Additionally, the high cost of treatment remains a major barrier, restricting access to a subset of eligible patients. In conclusion, monoclonal antibody therapy represents a significant milestone in the treatment of Alzheimer's disease, offering moderate yet meaningful benefits in carefully selected patients. However, stringent eligibility criteria, ARIA risk, continuous MRI surveillance, and high treatment costs pose substantial challenges to widespread adoption. While these therapies mark a critical advancement in Alzheimer's disease management, their long-term clinical impact and cost-effectiveness remain under investigation. In dementia with Lewy bodies, treatment is tailored to the main symptom domains. For cognitive symptoms, acetylcholinesterase inhibitors are first-line, with rivastigmine considered the most effective for improving cognition and neuropsychiatric features. Parkinsonism is treated cautiously with dopaminergic drugs such as levodopa, administered in low doses because of the risk of worsening hallucinations or psychosis. For psychotic symptoms, pimavanserin is preferred due to its favorable side effect profile, while quetiapine or clozapine may be considered if needed. Risperidone can also be used in selected cases, and unlike most other typical antipsychotics it has minimal effects on the cholinergic system, though it should still be prescribed with caution due to risks of motor worsening. Depression is common and is best treated with SNRIs or mirtazapine, the latter being particularly useful if there are concurrent sleep or appetite problems. Sleep disturbances, especially REM sleep behavior disorder, are managed with melatonin or clonazepam, and mirtazapine may also be beneficial. Non-pharmacological measures, such as strict sleep hygiene, should be combined with pharmacotherapy. In frontotemporal dementia (FTD), there is currently no approved disease-modifying treatment, though research is actively exploring antisense oligonucleotides targeting pathogenic proteins such as tau and TDP-43. Management is therefore mainly symptomatic. For behavioral and emotional disturbances, serotonergic agents such as SSRIs (e.g., sertraline, citalopram) and trazodone are commonly used, as they may reduce disinhibition, impulsivity, and compulsive behaviors. Glutamatergic drugs, particularly memantine, have been tried with variable results but may provide some benefit in selected patients. In cases of primary progressive aphasia, certain acetylcholinesterase inhibitors, such as galantamine, have shown limited clinical improvements, although their routine use in FTD is not established. In vascular dementia, therapy is primarily focused on controlling vascular risk factors to prevent further cerebrovascular injury. Strict management of hypertension, diabetes, and hyperlipidemia is essential, alongside lifestyle interventions such as smoking cessation and regular physical activity. Antithrombotic agents, including aspirin or clopidogrel, as well as statins, are commonly prescribed to reduce the risk of recurrent ischemic events. In selected patients, particularly those with mixed pathology involving Alzheimer's disease, acetylcholinesterase inhibitors and memantine may provide cognitive benefits. Although current pharmacological therapies are largely symptomatic rather than disease-modifying, timely and accurate diagnosis is a prerequisite for maximizing their effectiveness. Early and precise identification of the dementia subtype allows clinicians to choose the most appropriate treatment strategy, avoid harmful drug effects, and provide meaningful improvements in cognition, behavior, and quality of life.

WILL CLINICAL PHARMACOLOGY MAKE DRUG DEVELOPMENT SUSTAINABLE? A PROSPECTIVE VIEW.

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In 1987 a Phase I trial could be done for about 40.000 euros. The same trial today costs 100 times more. Has clinical pharmacology managed to increase the information content of these studies by a similar amount? There are now about 19000 prescription medicines available and the amount of instruction about pharmacology and therapeutics in medical schools hardly increased since the 1950's when there were perhaps hundred. Did the efficiency of the education, generally by clinical pharmacologists, increase proportionally? If this is not the case, there will be clear implications for the future and a call to action overdue.

EPHAR LECTURE 2025: DELIVERING EFFECTIVE PHARMACOLOGY EDUCATION: EVOLVING FROM THE LECTURE THEATRE TO ARTIFICIAL INTELLIGENCE

Maxwell S.[1]

1 University of Edinburgh, UK

The traditional model of pharmacology education, heavily reliant on lecture-based delivery, is undergoing a transformative shift towards integrating artificial intelligence (AI) technologies. This talk will explore the evolution of pharmacology education from static, lecture-based formats to dynamic, AI-enhanced methodologies. It will examine the limitations inherent in traditional educational models, such as passive learning, limited student engagement, and the challenge of addressing diverse learning needs in large cohorts. It will then explore the innovative use of AI in pharmacology education, highlighting specific technologies such as adaptive learning platforms, AI-driven simulations, and personalised learning experiences. AI tools not only have the potential foster a more interactive learning environment but also to enhance the understanding of complex pharmacological processes and drug interactions through sophisticated visualisations and real-time feedback. The talk will present case studies where AI has been successfully integrated, with improvements in student outcomes and engagement. Finally, it will address the challenges and ethical considerations of implementing AI in educational settings, including data privacy, the potential for bias in AI algorithms, concerns about assessment approaches and the need for maintaining an effective human-teacher presence. This talk aims to provide an overview of the future direction in pharmacology education, emphasising the role of AI in creating more effective, personalised and engaging learning experiences.

SOMATOSTATIN SST4 RECEPTOR IS A NOVEL TARGET IN THE THERAPY OF CHRONIC PAIN CONDITIONS: FROM EARLY DISCOVERIES TO CLINICAL TRIALS

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The treatment of chronic neuropathic pain is an unsolved clinical problem. Traditional analgesics have limited efficacy, hence the discovery of new targets is required. Our group has demonstrated that somatostatin, released from capsaicin-sensitive peptidergic sensory nerve terminals, mediates analgesic and anti-inflammatory effects via the somatostatin receptor subtype 4 (SST4), therefore the SST4 receptor selective agonists may be an effective option for the treatment of chronic pain. In this study, the receptor binding and activation properties of four small molecules were compared by drug-likeness investigation, pharmacokinetic prediction, molecular docking calculation in silico as well as cAMP accumulation assay in vitro and their anti-hyperalgesic effects in the partial sciatic nerve ligation (PSNL) mouse model of traumatic neuropathy in vivo. Docking calculations showed that all compounds interact with the conserved ASP126 of SST4. The cAMP accumulation assay performed in SST4 or SST2 overexpressing Chinese Hamster Ovary (CHO) cells confirmed that all molecules are selective on the SST4 receptor. PSNL-induced mechanical hyperalgesia was reduced by all examined compounds. Their anti-hyperalgesic effects varied between 20-70%. These results suggest that the tested compounds could be potentially effective in the treatment of neuropathic pain, although their pharmacokinetic predicted properties require further investigation to determine their penetration into the central nervous system.. Further clinical trials will determine whether the SST4 receptor-selective, analgesic, anti-inflammatory drug will be added to the pharmacotherapeutic palette. Supported by NKFIH K-134214, National Laboratory for Drug Research and Development, HUNREN.

RADIONICE / WORKSHOPS

INNOVATIONS IN PHARMACOLOGY AND MEDICAL EDUCATION

Guilding C[1], **Maxwell S.**[2]

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Pharmacology education is rapidly evolving to meet the demands of modern healthcare. This interactive workshop will explore innovative strategies for curriculum design, including integrated and spiral approaches that promote progressive learning and clinical application. We will also examine active learning methods such as role-play, virtual patients, and in-class polling, that enhance engagement and decision-making skills, alongside opportunities for interprofessional collaboration. Through small-group activities, participants will develop practical plans for implementing and evaluating these innovations in their own contexts, leaving with actionable ideas to advance pharmacology teaching and learning.

HOW TO DETERMINE THE HIGHEST PERMITTED WHOLESALE PRICE OF A MEDICINE IN CROATIA? – FROM APPLICATION TO DECISION.

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1 Agency for Medicinal Products and Medical Devices of Croatia (HALMED), Zagreb, Croatia

Determining the highest permitted wholesale price of a medicine in Croatia can be a complex process, often confronted with uncertainties in the interpretation of the regulatory framework. This workshop aims to provide a clear and practical overview of the relevant legal requirements and procedures. The workshop is based on the current Croatian legislation, primarily the Ordinance on the criteria for determining the highest permitted wholesale price of a medicine and exceptionally higher prices and annual price calculation. Using real-life examples, the workshop will guide participants through the entire process — from submitting an application for price calculation to receiving a final decision. Participants will gain a clear, systematic understanding of the methodology used to evaluate proposed prices. This includes the process of determining the maximum allowed price of a medicine, whether through external price referencing or a substantiated price proposal based on the manufacturer's price, as well as the procedures for requesting exceptionally higher prices and the notification process for price adoption. By clarifying key regulatory criteria and procedural steps, the workshop will support interested parties in preparing accurate and compliant pricing applications, ultimately contributing to a more transparent and predictable pricing process.

PHARMACOVIGILANCE - FROM THEORY TO PRACTICE

Kovacic Bytyqi B[1], Kulis I[1], Pavicic M.[1]

1 Agency for Medicinal Products and Medical Devices of Croatia (HALMED), Zagreb, Croatia

Risk minimisation measure (RMM) is defined as an intervention intended to prevent or reduce the occurrence of adverse reactions associated with the exposure to a medicinal product, or to reduce their severity or impact on the patient should an adverse reaction occur. RMMs can be categorised into routine and additional RMMs (aRMMs). Routine RMMs apply to every medicinal product, while aRMMs should be required only if they are considered necessary for keeping the risk-benefit balance of the medicinal product positive. Aim of this workshop is to raise awareness of aRMMs importance, discuss in which cases aRMMs should be required, and how they should be implemented. Valproate-containing medicinal products will serve as a practical example of aRMMs implementation. These medicinal products are used to treat epilepsy and bipolar disorder, and in some EU Member States, are also authorised to prevent migraine headaches. Risks of valproate use during pregnancy are congenital anomalies and developmental disorders in children exposed to valproate in utero. Additional RMMs are Pregnancy Prevention Programme (PPP), Direct Healthcare Professional Communication (DHPC) and Educational Materials including Guide for Healthcare Professionals, Patient Guide and Patient Card for both female and male patients, and Annual Risk Acknowledgement Form. Potential challenges of aRMMs implementation for these medicinal products will be discussed. At the end of this workshop, attendees will be able to recognise in which cases there is a need for aRMMs implementation, to define target population and most appropriate aRMMs tools for risk minimisation.

TRANSTHYRETIN STABILIZATION AS A PLEIOTROPIC NEUROPROTECTIVE STRATEGY: BRAIN-TARGETED KINETIC STABILIZATION MODULATES INFLAMMATION AND ANTIOXIDANT PATHWAYS IN NEURODEGENERATION

Babic Perhoc A[1], Homolak J[1,2], Pavic K[3], Knezovic A[1], Virag D[1], Osmanovic Barilar J[1], Salkovic-Petrisic M.[1]

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The global burden of neurodegenerative disease underscores the urgent need to improve therapeutic outcomes, mandating a shift towards targeting the early pathophysiological, coupled with identification and amplification of the body's intrinsic neuroprotective mechanisms. Transthyretin (TTR) is a transport protein primarily synthesized in the liver and choroid plexus, with a well-established role in binding and stabilizing thyroxine and retinol- binding protein, and known for causing different types of systemic amyloidosis, primarily affecting the heart and nerves. In the context of Alzheimer's disease (AD), TTR has been shown to interact with Aβ, preventing its aggregation and promoting its clearance, suggesting a potential neuroprotective function. Our preliminary findings suggest that early upregulation of TTR in the initial stages of neurodegeneration might play an important protective role through its diverse, pleiotropic functions, particularly by regulating inflammation and metabolic homeostasis. By modulating multiple pathways involved in neuroprotection, TTR helps counteract disease progression. Given the critical role of inflammation and metabolic regulation in other neurodegenerative disorders, our findings indicate that TTR's protective effects extend beyond AD and may also be beneficial in conditions with shared pathophysiological mechanisms, such as Parkinson's and multiple sclerosis. Our team has successfully engineered a modified TTR kinetic stabilizer for brain delivery, demonstrating for the first time that benefits of TTR stabilization go beyond the effects on Aβ clearance. This novel, brain-permeable TTR stabilizer applied in a mouse model of AD successfully reduced neuroinflammation and robustly upregulated antioxidant gene expression in the hippocampus, even in the absence of changes in amyloid burden.

CHALLENGES IN TEACHING BASIC PHARMACOLOGY FOR PHARMACY STUDENTS

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Pharmacology is a core subject in the fourth year of the pharmacy diploma program at the University of Zagreb Faculty of Pharmacy and Biochemistry, delivered through Pharmacology 1 and 2 courses. The curriculum includes 75 hours of lectures, 45 hours of seminars, and 15 hours of experimental work. Over the past decade, the curriculum has undergone modifications, particularly in the experimental component. An anonymous questionnaire was used to gather student feedback. Participants rated their (dis)agreement with various statements related to lectures, seminars, and experimental work using a Likert scale and provided open-ended comments with suggestions for improvement. Students value clear explanations, interactive teaching, real-life examples, well-defined learning outcomes, and informative presentations. Problem-solving activities during seminars and short quiz questions were particularly appreciated for reinforcing knowledge. The modified experimental exercise—highlighting challenges in experimental pharmacology and ethical considerations around animal use, supplemented with video materials—was rated as beneficial for deepening students' understanding of pharmacological research. Despite the challenges in teaching basic pharmacology, the continuous implementation of innovative teaching methods—particularly within a stimulating, team-based learning environment—can provide students with a comprehensive understanding of pharmacological principles essential for their future professional practice and academic development.

CHALLENGES IN THE TREATMENT OF PAIN AND THE DEVELOPMENT OF NEW ANALGESICS

Bach-Rojecky L.[1]

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The management of both acute and chronic pain remains a largely unmet public health challenge. Nearly one in five adults in the European Union suffer from chronic pain that significantly interferes with daily activities. Pain therapy commonly relies on two large analgesic drug classes – opioids and non-steroidal anti-inflammatory drugs, with several adjunctive analgesics from other pharmacotherapeutic groups (like some antiepileptics and antidepressants). However, current pain therapies often face limitations such as inadequate efficacy, unacceptable safety profiles, or restricted accessibility. This overview examines current pharmacotherapeutic options for acute and chronic pain management through a review of recent scientific literature, ongoing late-stage clinical trials, and analgesic drug development pipelines. After nearly 25 years, suzetrigine—a first-in-class drug acting as a selective peripheral Nav1.8 channel inhibitor—was approved by the FDA in 2025 for the treatment of acute pain. Currently, several investigational analgesic options are in various stages of clinical development, including fixed-dose combinations designed to simultaneously target multiple pain pathways, as well as co-crystal formulations that employ co-crystal technology to optimize the physicochemical and pharmacokinetic properties of combined drugs. In addition to oral formulations, a range of innovative transdermal and other parenteral delivery systems are also being explored. The effective management of acute and chronic pain necessitates innovative therapeutic strategies aimed at enhancing efficacy, improving safety profiles, and minimizing the potential for drug abuse. These objectives may be achieved through the development of novel analgesic agents, drug repurposing, advanced drug formulations, and rationally designed fixed-dose combinations.

MEDITERRANEAN DIET: WITH OR WITHOUT WINE?

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The Mediterranean diet is considered one of the healthiest dietary patterns in the world. There is a huge amount of scientific literature which practically unanimously indicate that Mediterranean-like dietary patterns are beneficial in preventing and controlling diet-related non-communicable diseases such as diabetes, cardiovascular diseases, neuro-degenerative diseases and cancer. The World Health Organization has also identified the Mediterranean diet as an effective dietary strategy to prevent non-communicable diseases, which are the leading cause of premature death globally. Studies examining the relative importance of the individual components of the Mediterranean diet to its association with lower all-cause mortality, found that the dominant component of the Mediterranean diet as a predictor of lower mortality was moderate consumption of alcohol, particularly in the form of red wine. On the other hand, from time-to-time population studies emerge with the message that any consumption of an alcoholic beverage is harmful to health, calling for practically complete abstinence for all. Hence, recommendations to adopt Mediterranean diet but avoid any alcoholic beverage may be confusing for both general population and medical professionals. In this presentation it will be discussed the main sources of disagreements between different publications and documents dealing with the relationship between alcohol and health.

DRUG-DRUG-GENE INTERACTIONS IN PREDICTING CARDIOVASCULAR DRUG-RELATED EFFICACY AND SAFETY OUTCOMES

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In cardiovascular drug-related efficacy and safety outcomes, concomitant medications (drug-drug interactions, DDIs) and genomic factors (drug-gene interactions, DGIs) have a profound influence. Project PGx-CardioDrug investigates cumulative effects of DDIs and DGIs (DDGIs) on efficacy and adverse drug reaction risk (ADRs) in patients prescribed direct oral anticoagulants (DOACs), platelet aggregation inhibitors (PAIs), and statins. The cohort included subjects with prescribed DOACs, PAIs and/or statins. Cases were subjects experiencing ADRs during follow-up: bleeding/inefficacy for DOACs/PAIs, myotoxicity/hepatotoxicity for statins, and other ADRs; controls were patients without ADRs. Relevant gene variants were genotyped by TaqMan real time PCR: CYP2C9, CYP2C19, CYP2C:TG haplotype, CYP2D6, CYP2J2, CES1, CYP3A4, CYP3A5, ABCB1, ABCG2, SLCO1B1, according to therapy. Clinical and laboratory parameters were monitored, ADRs assessed, and DDIs evaluated using Lexicomp®. Of 1,911 recruited subjects (1,234 statins, 773 DOACs, 294 PAIs) 1,433 were analysed. Observed statin ADRs - atorvastatin (154/606), rosuvastatin (185/620): rhabdomyolysis (8 cases), myotoxicity (19 21%), hepatotoxicity (6 7%), other (4 9%). DOAC ADRs (141/773): rivaroxaban (74/410: bleeding=58, inefficacy=16), apixaban (25/194: bleeding=17, inefficacy=7), dabigatran (36/113: bleeding=26, inefficacy=9). PAIs ADRs (32/294: bleeding=20, inefficacy=12). Potential DDI with increased risk: statins (level C=15%; D=2.2%), DOACs (level C=21%, D=26%), PAIs (level C=71%, D=2.6%). DGI associations: DOACs not significant with ABCB1, clopidogrel trend with CYP2C19/CYP2C:TG, rosuvastatin significant with ABCG2/SLCO1B1, atorvastatin significant with SLCO1B1 and trend with ABCG2/CYP3A4. DDGI: rivaroxaban no association with ABCB1, clopidogrel no association with ABCB1/CYP2C19, atorvastatin trend for CYP3A4. Our preliminary data point to the drug-drug-gene interactions as a potential risk factor for cardiovascular drugs adverse reactions.

PRECLINICAL NEUROIMAGING IN DRUG DISCOVERY

Cash D.[1]

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Drug discovery for brain disorders faces persistent challenges, including the inaccessibility of the brain, ethical and financial barriers to human testing, and the limited translational value of animal models. Neuroimaging offers a powerful, non-invasive, and translatable tool to bridge this gap, but preclinical imaging approaches often suffer from a lack of standardisation. In this talk, Dr Diana Cash—Associate Professor and Scientific Director of The Brain Centre at King's College London—will present a novel strategy for enhancing the translational impact of preclinical imaging through pharmacological fingerprinting. Her team is building a reference database of neuroimaging "fingerprints" from well-characterised neuroactive drugs using functional MRI (BOLD and ASL) in rodents. These fingerprints can serve as benchmarks for evaluating new compounds, offering insights into their mechanisms of action and potential clinical relevance. The presentation will highlight core imaging methodologies, translational considerations between rodent and human studies, and findings from the FFIND (Functional Fingerprinting in Neuroactive Drugs) project. This dataset includes six compounds—ketamine, MK801, psilocybin, donepezil, clozapine, and levetiracetam—chosen for their diverse pharmacological profiles. The goal is to accelerate and refine the drug development process by improving early-stage screening and reducing the attrition of candidate therapeutics. By advancing the standardisation and interpretability of preclinical neuroimaging, this work aims to strengthen the bridge from bench to bedside and support the discovery of more effective treatments for neurological and psychiatric disorders.

READING AND INTERPRETING AN IMPD/IB. A STRUCTURED METHOD AND A DIGITAL TOOL

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1 Centre for Human Drug Research and Leiden University Medical Centre, Leiden, the Netherlands

Research with new medicines is a multidisciplinary affair. Particularly in early phase research the assessment of the available preclinical information is essential to estimate the risk for participants and adequate dosage. The preclinical information covers a broad area and is presented in an 'Investigator's Brochure' (IB) or in the Investigational Medical Product Dossier (IMPD). These documents have a considerable size and complex content. There are experiments in a range of animal species, and ex-vivo models, toxicological experiments, and dosage in metric or molar units. Most importantly there rarely are tables combining the findings. This lack of a standardised dashboard makes wrong or biased decisions more likely. We developed a digital – open source – tool for summarising the data in relation to expected pharmacodynamic and toxic effects based on known pharmacokinetic parameters. This tool will be demonstrated during the lecture. The tool is freely available from ib-derisk.org.

NEW DEVELOPMENTS IN THE PHARMACOLOGICAL MANAGEMENT OF SLEEP DISORDERS IN DEMENTIA

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With over three decades of clinical insight into sleep medicine, the evolution of pharmacological approaches to sleep disorders in dementia reveals both remarkable progress and persistent complexity. Sleep disturbances ranging from insomnia and circadian rhythm disorders to REM sleep behavior disorder (RBD), hypersomnolence, and sleep-disordered breathing (SDB) are not only prevalent in dementia syndromes, but often precede cognitive decline, suggesting a deeper pathophysiological interplay. This presentation critically reviews emerging therapeutic strategies based on both clinical experience and recent translational findings. Notably, dual orexin receptor antagonists (DORAs) offer promising efficacy in sleep initiation and maintenance with minimal cognitive burden, while the role of melatonin and its agonists continues to evolve in circadian rhythm regulation, particularly in Alzheimer's and Lewy body dementia. The nuanced use of serotonergic and dopaminergic agents in RBD, especially within alpha-synucleinopathies, underscores the need for individualized treatment paradigms. Importantly, untreated SDB, especially obstructive sleep apnea (OSA), represents a modifiable risk factor for cognitive decline, with growing evidence linking OSA-related intermittent hypoxia and sleep fragmentation to accelerated neurodegeneration. Timely recognition and intervention, whether with positive airway pressure (PAP) therapy or emerging pharmacological adjuncts, may mitigate cognitive deterioration in this vulnerable population. Despite its high prevalence, OSA remains underdiagnosed in dementia care, warranting greater integration of sleep diagnostics into memory clinics and geriatric practice. Neuropharmacological advances, supported by fluid biomarkers and functional imaging, are reshaping how we stratify sleep-related phenotypes in dementia. These tools enable more precise targeting of disrupted neurotransmitter systems and may soon allow earlier pharmacological intervention. Nevertheless, gaps remain, particularly regarding long-term safety, polypharmacy risks, and the pharmacokinetics of commonly used agents in aging brains with neurodegeneration. This overview bridges bench research with decades of bedside observations, advocating for an integrative, evidence-based, and patient-centered approach to treating sleep disorders in dementia.

PSYCHOPLASTOGENS AS NOVEL MODULATORS OF NEUROINFLAMMATION

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A new therapeutic paradigm has emerged in neuropsychiatry and neurology, centered on psychoplastogens, a class of compounds capable of rapidly promoting structural and functional neuroplasticity. This group includes classical psychedelics (e.g. psilocybin, LSD, DMT), ibogaine, dissociative anesthetics (e.g. ketamine), MDMA, and newer synthetic compounds lacking hallucinogenic effects (e.g. tabernanthalog). Beyond their psychotropic effects, psychoplastogens demonstrate potent anti-inflammatory and neuroprotective properties that position them as promising candidates for treating glia-mediated neuroinflammation in neurodegenerative diseases. Recent studies reveal that psychoplastogens exert their effects primarily through serotonin 5-HT2A receptor activation, with downstream modulation of key signaling pathways such as NFκB, PI3K/Akt, and mTOR. These pathways regulate microglial activation, cytokine production, and synaptic remodeling. Notably, psychoplastogens also influence non-serotonergic systems like glutamatergic, dopaminergic, GABAergic, and can modulate the kynurenine pathway, shifting the balance toward neuroprotective metabolites. Natural compounds like ayahuasca have demonstrated reductions in neuroinflammatory markers and oxidative stress in preclinical models, while novel non-hallucinogenic analogs have potential to maintain therapeutic efficacy with improved safety profiles. Clinical studies support the efficacy of psychoplastogens in depression, PTSD, and substance use disorders, conditions often marked by neuroinflammation, while their application in neurodegenerative diseases is an expanding frontier. Next to the therapeutic efficacy, current evidence indicates their low potential for dependence, minimal toxicity, long-lasting therapeutic effects from a single administration, and broad treatment potential. Here, the mechanistic underpinnings and translational potential of psychoplastogens as multi-target modulators of glial activity and chronic inflammation will be presented, offering a paradigm shift in the treatment of CNS disorders.

OVERVIEW OF NON-CLINICAL SAFETY EVALUATION OF NEW ACTIVE SUBSTANCES

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The non-clinical safety evaluation of new active substances (NAS) is a fundamental component of medicine development, aimed at identifying potential adverse effects before initiating clinical trials. This process encompasses a comprehensive battery of in vitro and in vivo studies designed to assess pharmacological activity, dose-response relationships as well as the safety pharmacology (effects on vital organ systems such as the cardiovascular, respiratory, and central nervous system) and general toxicological profile, to establish safe starting doses for first-in-human (FIH) studies. In parallel with clinical trials, toxicological profile is further characterized in a greater extent to identify potential risks to human health following the intended marketing application. Core elements of non-clinical safety testing include repeat-dose toxicity studies, genotoxicity, carcinogenicity and reproductive and developmental toxicity. Additional specialized studies may be required depending on the nature of the NAS. Regulatory guidelines provide standardized frameworks for conducting these evaluations, ensuring consistency and scientific rigor across global markets and promoting a science-based approach that balances between the unnecessary use of the animals (3Rs, i.e. Replacement, Reduction, and Refinement, principles) and patient safety. Species selection, dose range determination, and study duration are critical factors influenced by the intended clinical indication and duration of human exposure. Overall, non-clinical safety evaluation provides essential data for risk-benefit assessment and informs decision-making throughout the medicine development lifecycle. Relevant information from non-clinical studies is provided in section 5.3 of the summary of product characteristics (SmPC) which healthcare professionals may use to additionally recognize the safety profile of the substance.

TRIMETAZIDINE AND WOUND HEALING: TRANSCRIPTOMIC INSIGHTS INTO ANGIOGENESIS, INFLAMMATION AND METABOLISM IN DIABETIC FOOT ULCERS

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Diabetic foot ulcers (DFUs) are among the most debilitating complications of diabetes. They are characterized by impaired angiogenesis, persistent inflammation and disrupted cellular metabolism. Despite advances in revascularization and wound care, few pharmacological options directly target these molecular abnormalities. Trimetazidine (TMZ) is an established anti-ischemic agent with known vascular and metabolic benefits, but its potential role in wound healing biology remains insufficiently studied. We performed an in silico transcriptomic analysis using the Library of Integrated Network-Based Cellular Signatures (LINCS) Phase 5 dataset. TMZ-induced gene expression signatures were evaluated in four human cell models with relevance to DFU pathophysiology: HUVEC (endothelial), THP1 (monocytic), A375 (keratinocytic-like) and MCF7 (epithelial-like). Differentially expressed genes were identified using standardized z-score thresholds, followed by over-representation and gene set enrichment analyses to uncover altered pathways. To assess disease relevance, results were cross-validated using an independent bulk DFU tissue dataset (GSE199939). TMZ induced between 700 and 900 differentially expressed genes per cell line. Despite cell-type specific responses, several convergent biological processes emerged. PI3K–Akt signaling was consistently upregulated. Noncanonical NF-κB components such as RELB and CXCL2 were modulated. TMZ promoted metabolic reprogramming with SIRT3 upregulation and suppression of glycolytic enzymes, suggesting enhanced mitochondrial efficiency under hypoxic conditions. Extracellular matrix remodeling genes, including SPP1 and LAMA3, were selectively regulated, supporting structural repair. Importantly, these pro-healing signatures developed largely independently of VEGF, with alternative angiogenic mediators such as CXCL2 and NOTCH1 prominently involved. Cross-validation in DFU tissue confirmed enrichment of PI3K–Akt, NF-κB, oxidative phosphorylation and extracellular matrix–receptor pathways. SIRT3 was significantly downregulated in DFU tissue, consistent with mitochondrial dysfunction. TMZ induces a coordinated transcriptomic program that converges on angiogenesis, inflammation resolution and metabolic adaptation, all of which are critical for wound healing. These exploratory findings support TMZ as a potential repurposed therapy for DFUs and provide a rationale for preclinical validation and translational studies.

UNLOCKING THE POWER OF INHIBITING SOLUBLE EPOXIDE HYDROLASE AND SIGMA-1 RECEPTOR ANTAGONISM THROUGH DUAL MECHANISM ANALGESIC COMPOUNDS

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Pain affects over 40% of the global population, but current therapies often lack effectiveness. Innovative analgesics with better efficacy, fewer side effects, and lower abuse potential are urgently needed. With a pressing need for non-abusable analgesics, recent research has identified new potential targets. This project aims to develop a first-in-class dual analgesic compound to address the management of pain. Our strategy centers on the design of compounds that inhibit soluble epoxide hydrolase (sEH) and antagonize the sigma-1 receptor (SIR). Inhibition of sEH enhances epoxyeicosatrienoic acids, showing beneficial therapeutic effects in neuropathic pain. Moreover, SIR is involved in pain signaling, and preclinical studies suggest that its antagonism may relieve hypersensitivityl. Of relevance, we recently discovered that the concurrent modulation of both targets amplifies the analgesic effects, offering a synergistic approach to alleviating pain. Therefore, our group firstly designed, synthesized and biologically evaluated a series of compounds and performed a structure–activity relationship study of the left-hand side of the molecule. Next, as disclosed in the present work, a hit-to-lead optimization of the right-hand side of the molecule has been performed. Herein, we present the first dual acting compounds that, in addition to inhibiting sEH in the low nanomolar range, have a low nanomolar affinity for the SIR. After performing a screening cascade, ten compounds emerged as the most promising, displaying favorable characteristics, such as good DMPK properties in in vitro assays, and were selected for subsequent in vivo assays. Interestingly, several compounds were endowed with a potent anti-allodynic effect in the well-established capsaicin-induced murine model of allodynia and the postoperative pain model.

MULTIFUNCTIONAL COMPOUNDS FOR POTENTIAL TREATMENT OF ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is characterized by cholinergic hypofunction and neuroinflammation, yet current therapies address only single pathological mechanism. Butyrylcholinesterase (BChE) activity increases with AD progression, while overactivation of p38α mitogen-activated protein kinase (p38α MAPK) by amyloid beta (Aβ) plaques exacerbates neuroinflammation and cognitive decline. Simultaneous inhibition of BChE and p38α MAPK represents a novel therapeutic strategy for AD. We used structure-based drug design to develop dual-acting small molecules targeting both BChE and p38α MAPK. The most promising compounds were characterized by X-ray crystallography and evaluated in vitro, ex vivo, and in vivo using enzyme assays, neuroinflammation models, and cognitive behavioral tests in mice. Two lead compounds demonstrated balanced dual inhibition of BChE and p38α MAPK, confirmed by crystallographic studies. In cell-based assays, these compounds reduced proinflammatory cytokine production. In vivo, they improved cognitive performance in scopolamine- and LPS-induced mouse models of cognitive impairment and reduced brain inflammation. Notably, their efficacy in cognitive tasks surpassed that of reference drugs rivastigmine and neflamapimod. Our dual BChE/p38α MAPK inhibitors represent a promising multifunctional approach for AD therapy, potentially addressing both cholinergic deficits and neuroinflammation, and providing superior efficacy compared to single-target treatments.

MONITORING TIME-DEPENDENT BRAIN DAMAGE AND REPAIR AFTER ISCHEMIC INJURY IN LIVING ANIMALS AS A TOOL FOR EVALUTION OF SIGMA-1 RECEPTOR RELATED PRECLINICAL INTERVENTIONS

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Stroke represents a major health problem, and its ischemic variant is the most prevalent. The current interventions are based on recanalization of the affected blood vessels. However, the neuroprotective and neurorestorative strategies remain elusive despite a significant body of research in this direction. To address this issue, we have arranged a comprehensive preclinical platform to monitor the damage and repair processes after ischemic brain injury on living animals. As clinically relevant model, transient middle cerebral artery occlusion (tMCAO) was used to achieve ischemia reperfusion injury corresponding to ischemic stroke successfully treated by recanalization. Subsequently, structural, cellular, and functional consequences of stroke were monitored by in vivo imaging and functional tests. The structural brain changes were assessed by in vivo magnetic resonance imaging (MRI). This allowed to measure the volume of ischemic injury at successive time points until its consolidation and final brain tissue loss, which we estimated to occur around 4 weeks after tMCAO. The cellular processes in the brain were visualized by in vivo bioluminescence imaging (BLI), using the collection of transgenic animals where luciferase expression was driven with specific promoters. The most used transgenic mice is TLR2-luc, where TLR2 promoter was used resulting with imaging of neuroinflammation. The functional stroke consequences are monitored by behavioral tests resulting in a neurological score. All these parameters can be correlated between each other, the rates of change measured, and stroke damage and repair adequately modelled. Sigma-1 receptors are involved in both brain damage after ischemic injury and the subsequent repair. In conclusion, the described system of evaluating brain damage and repair represents a useful way to get insight in the dynamic of Sigma-1 receptor activities and to evaluate the effects of preclinical interventions based on modulating Sigma-1 receptor effects.

DPYD GENOTYPING: WHICH GENE VARIANTS SHOULD BE INCLUDED IN DOSING ALGORITHMS?

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Pharmacogenomics (PGx) testing is part of precision medicine that enables the improvement of pharmacotherapy efficacy and safety. Fluoropyrimidines (FPs), including 5 fluorouracil and capecitabine, are widely used antineoplastic agents for various solid tumors. However, 20 30% of patients treated with FP chemotherapy experience severe toxicity, and in up to 1% of treated patients, toxicity can be fatal. Patients with reduced activity of dihydropyrimidine dehydrogenase (DPD), the main enzyme responsible for the breakdown of FP, are at an increased risk of experiencing severe FP-related toxicity. The European Medicines Agency (EMA) recommends testing DPD deficiency, using either phenotyping or genotyping, before starting treatment with FPs. Several pharmacogenomic consortia (such as CPIC, DPWG, and RNPGx) recommend pre treatment testing by genotyping specific loss of function or reduced function DPYD gene variants to reduce the risk of severe FP toxicity. Current guidelines highlight dosage adjustments with four clinically relevant DPYD variants: DPYD *2A (rs3918290), *13 (rs55886062), c.2846A>T (rs67376798), and HapB3/c.1236G>A (rs75017182/rs56038477). However, this four-variant approach fails to identify all at-risk individuals, suggesting that additional genetic factors contribute to DPD deficiency and FP toxicity. Recent research highlights the potential of including additional common and rare DPYD variants, such as c.496A>G, to further improve risk prediction. The broader use of next-generation sequencing (NGS) and improved functional characterization of novel and rare variants are necessary for a more comprehensive clinical implementation. Standardization of genotype phenotype translation and a better understanding of the impacts of common and rare variants are essential for addressing the “missing heritability” of DPD deficiency and optimizing PGx-guided FP therapy.

EXERCISE PRESCRIPTION AS PART OF THE TREATMENT PLAN FOR CHRONIC DISEASES

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More than 70% of deaths worldwide are caused by chronic non-communicable diseases (NCDs), which include heart disease, type 2 diabetes, and several types of cancer. Lack of physical activity is one of the main modifiable risk factors causing NCDs. Physical inactivity is nevertheless common in all age categories, despite ample evidence of its advantages. Research confirms that regular physical activity reduces the risk of developing and progressing numerous chronic diseases, as exercise positively affects metabolic control, cardiovascular function, inflammation, and mental health. Therefore, physical activity represents a well-established evidence-based intervention with both preventive and therapeutic effects across a wide spectrum of NCDs. International guidelines recommend that adults engage in at least 150–300 minutes per week of moderate-intensity aerobic activity, or 75–150 minutes of vigorous-intensity activity, combined with muscle-strengthening activities on two or more days per week. For individuals with chronic conditions, physical activity should be adapted to functional capacity and health status, aiming to reduce sedentary behavior and gradually progress toward guideline targets. Integration of structured exercise prescription into routine clinical practice necessitates that healthcare professionals are adequately trained to assess physical activity levels, formulate individualized exercise programs, and monitor patient adherence and outcomes. The implementation of standardized physical activity guidelines within multidisciplinary treatment frameworks offers a scalable, cost-effective, and accessible strategy to mitigate the global burden of chronic diseases.

ADVANCED THERAPY MEDICINAL PRODUCTS

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Advanced therapy medicinal products (ATMPs) represent an innovative class of biopharmaceuticals offering new therapeutic options across a range of diseases. ATMPs are classified as gene therapy medicinal products (GTMP), somatic cell therapy medicinal products (sCTMP), tissue engineered products (TEP) or combined ATMPs (containing medical device(s) as integral part). The common legal and regulatory framework for ATMPs in the EU was established by Regulation (EC) 1394/2007, which has been in force since 2008. Since then, EMA has published numerous ATMP-specific guidelines and developed early assessment tools to support and improve the success of ATMP marketing authorisation (MA) applications. Product development in ATMP field faces significant challenges due to their complex nature, and it differs substantially from the development of conventional medicinal products. The regulatory framework, high cost of development, translation from research to clinical trials and into clinical practice still represent key obstacles that make ATMP MA very challenging. Following the implementation of the ATMP Regulation, 28 products have been authorised in EU, of which 20 remain on the market: 1 sCTMP, 17 GTMPs and 2 TEPs. The dominance of GTMPs reflects significant technological advances and growing scientific and commercial interest, particularly following the EU approvals of CAR-T cell therapies Kymriah and Yescarta in 2018. Furthermore, in 2024, MA was granted to Casgevy, the first-in-class GTMP comprising ex vivo CRISPR/Cas9-edited genetically modified cells, marking a major milestone in gene editing therapies. A robust ATMP pipeline suggests that many candidates are expected to apply for EU marketing authorisation in the near future.

DEVELOPMENT OF SILICON-BASED, CAPSAICIN AND DICLOFENAC CONTAINING TRANSDERMAL PATCHES AGAINST NEUROPATHIC PAIN CONDITIONS

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Persistent nociceptive and neuropathic pain remains refractory to the drug treatment. We evaluated a novel silicone-based transdermal therapeutic system (TTS) co- formulated with low-dose capsaicin and diclofenac, characterizing its multimodal analgesic efficacy in four rat models: post-incisional, carrageenan-induced inflammatory, partial sciatic nerve ligation (PSNL) and monosodium iodoacetate (MIA)-induced osteoarthritis. In vitro Franz diffusion and flow-through cell assays determined capsaicin and diclofenac release, confirming near zero-order kinetics and mutual permeation enhancement. Acute post-incisional and carrageenan models were assessed by increased-temperature water bath for thermal hyperalgesia and dynamic plantar aesthesiometry (DPA) for mechanical allodynia at baseline, post-induction, and 2.5 h and 6 h after TTS application (capsaicin 0.39 mg/cm^2; diclofenac 0.39 mg/cm^2; combination 0.38 + 0.38 mg/cm^2; placebo). Chronic PSNL was evaluated on days 7, 14 and 21 via DPA-derived withdrawal thresholds. MIA- induced osteoarthritis was monitored on days 7 and 15 for mechanical allodynia, dynamic weight bearing and knee joint diameter. The dual-agent patch delivered continuous and zero-order kinetics. In acute models, the combination markedly reduced thermal and mechanical hypersensitivity at both 2.5 and 6 hours, outperforming monotherapies. In the PSNL model, mechanical thresholds were restored to near-baseline levels with combined and capsaicin-only formulations at 6h. In the MIA model, the dual patch alleviated joint pain, normalised load distribution and decreased articular swelling relative to single-agent treatments. This silicone-based transdermal system provides optimized, controlled-release capsaicin and diclofenac, achieving, prolonged analgesia and represents a promising candidate for clinical development in both inflammatory and neuropathic pain syndromes. Patent pending: P2200237 (27.06.2022.), PCT/HU2023/050042 (27.06.2023.), US 63/615,177 (27.12.2023.) Funding: TKP2021-EGA-13, Proof of Concept Grant, University of Pécs, 2024

INNOVATIONS IN EARLY PHASE CLINICAL DRUG STUDIES: ADVANCEMENTS IN NEUROLOGY AND PAIN

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Early phase clinical drug studies are critical for evaluating safety, pharmacokinetics, and pharmacodynamics (PD) of novel compounds. Demonstrating PD effects early can significantly de-risk drug development and optimize decision-making. Our center has developed and implemented innovative, in-house methodologies for early phase trials, among others in the therapeutic areas of neurology and pain. We present a series of early phase studies conducted at our center, focusing on novel CNS and analgesic compounds. These trials employed tailored experimental medicine approaches, including neurophysiological biomarkers, and PD assays. Our methods aimed to quantify target engagement and mechanistic effects in healthy volunteers and patient populations. Key case studies include: (1) A first-in-human study of a novel chloride channel blocking agent where an experimental neurophysiological method demonstrated mechanistic target engagement within single-dose escalation cohorts, which translated to clinical effects in patients with Myasthenia Gravis; (2) An evoked pain model study using a battery of nociceptive tests in which a dose dependent effect of a novel analgesic on capsaicin induced allodynia was demonstrated. Across studies, early PD signal detection enabled go/no-go decisions, dose optimization, and refinement of subsequent trial designs. Our findings underscore the value of integrating innovative PD assessments into early phase trials. These approaches not only enhance understanding of drug mechanisms but also increase the efficiency and success rates of clinical development programs. The methodologies presented can serve as practical models for academic and industry researchers aiming to bridge the translational gap in drug development.

CORE CONCEPTS OF PHARMACOLOGY EDUCATION: AN INTERNATIONAL COLLABORATION

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The IUPHAR Education Section (IUPHAR-Ed) is the global educational arm of the International Union of Basic and Clinical Pharmacology, dedicated to improving pharmacology education through international collaboration. Educators and graduates have raised concerns about gaps in graduates' abilities to apply pharmacology principles to clinical and research settings, compounded by the field's rapidly evolving knowledge base. In response, the IUPHAR-Ed Core Concepts of Pharmacology project (CCP) is developing a new approach to pharmacology education focussed on graduate understanding and application of the core knowledge of our discipline. Delphi studies and text mining were first used to identify the core concepts of pharmacology. These concepts were then further defined and unpacked using a two-phase iterative process. An international expert group of pharmacology educators developed draft definitions through online meetings and asynchronous collaboration. These drafts were refined during a two-day hybrid workshop, followed by continued online input. 24 core concepts and 103 sub-concepts of pharmacology were identified, defined and unpacked. More recently we have explored student understanding of the concepts and revealed common misconceptions. By the end of 2024 the CCP had involved over 400 educators and 1500 students from 28 countries. The outputs from the CCP research can be used to develop teaching and assessment resources which help students gain the conceptual knowledge they need to solve novel and complex problems related to the development and safe use of medicines. Continued coordinated research efforts will ensure that these resources are evidence-based and engage the global pharmacology community.

HUMAN BRAIN ORGANOIDS AS AN INNOVATIVE TOOL FOR EXPLORING NEUROINFLAMMATORY PATHWAYS AND TESTING NOVEL THERAPEUTICS IN NEURODEGENERATIVE DISEASES

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Neurodegenerative diseases are closely associated with chronic neuroinflammation, where glial cells play a pivotal role in modulating pathological processes. Human brain organoids represent an innovative model that enables three-dimensional investigation of neuron-glia interactions in conditions mimicking human brain. This approach offers a unique opportunity to explore neuroinflammatory pathways in detail and to test novel therapeutic strategies aimed at modulating glia-mediated neuroinflammation in neurodegenerative diseases. The initial phase of the research involves establishing a stable cell culture of human-induced pluripotent stem cells (iPSCs). Once a stable cell culture is established, the cells are grown in specialized media with supplements, such as growth factors, to generate human brain organoids. After approximately 30–45 days of cultivation and development of brain organoids in nutrient media, they reach a specific size suitable for further analysis. Inflammatory stimulation is applied to brain organoids to assess the levels of pro-inflammatory cytokines and markers of glial activation. Preliminary testing of selected therapeutic compounds then are proceeded to evaluate their effects on inflammation and neurodegeneration. Brain organoids can be used as new model organoids to research the development and various pathologies of the human brain. They imitate the main features of the early development of the human brain at the molecular, cellular, structural, and functional level, the more complex connection of neurons within the brain and the cellular signalling itself are yet to be researched. The application of brain organoids is highly useful for researching the development of neurodegenerative disorders.

NEUROTROPIC EFFECTS OF OXYSTEROLS – HARMFUL OR HARMLESS SIDE EFFECTS OF MEDICATION AFFECTING CHOLESTEROL SYNTHESIS?

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Oxysterols are oxidized derivatives of cholesterol or other sterols. Elevated levels of reactive oxygen species, cholesterol-rich diets, inflammation, disruption in cholesterol biosynthesis or degradation can lead to enzymatic or non-enzymatic addition of oxygen-containing groups to the sterol ring. Once formed, oxysterols act as important signalling molecules regulating cholesterol synthesis, modulating inflammation, influencing apoptosis and cell proliferation, and supporting/obstructing neuronal survival, neuritogenesis, myelination, and synaptogenesis. The separation of cholesterol synthesis in the brain from peripheral tissues results in a 10-15-fold higher accumulation of cholesterol in neural structures. However, this separation is not complete; several sterols and oxysterols, such as 24S-hydroxycholesterol and 27-hydroxycholesterol, can cross the blood-brain barrier (BBB). An analysis of the FDA-approved drug library by Wages et al. revealed that 20% of these drugs influence cholesterol synthesis by inhibiting the final enzyme, 7-dehydrocholesterol reductase (DHCR7), leading to increased levels of 7-dehydrocholesterol (7DHC) or affecting desmosterol levels. 7DHC readily converts to oxysterols and is implicated in the neurological symptoms of Smith-Lemli-Opitz syndrome. Notably, at least one-third of these drugs can cross the BBB. Since many of these medications (antihypertensives, antiarrhythmics, antihistamines, antipsychotics, cytostatics, statins) are frequently prescribed chronically to children and adults, their impact on cholesterol metabolism warrants attention. Of particular concern is the use of medications that influence 7DHC levels as polypharmacy or in individuals who are heterozygous for DHCR7 gene mutations. In these cases, it is to be expected that their iatrogenic effects may contribute to cholesterol-based pathophysiological mechanisms involved in human diseases, including atherosclerosis and Alzheimer's.

ALZHEIMER'S DISEASE INCREASES OXIDATIVE STRESS VULNERABILITY AND MAY CONTRIBUTE TO DISEASE PROGRESSION

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Transthyretin (TTR) is a multifunctional protein with neuroprotective properties. In murine models of sporadic Alzheimer's disease (sAD) induced by intracerebroventricular streptozotocin (STZ-icv), leptomeningeal amyloid deposits resembling TTR amyloidosis have been observed. This study aimed to investigate the involvement of TTR in these deposits and its potential role in modulating oxidative stress susceptibility. Male C57BL/6 mice received STZ-icv (6 mg/kg; 1 µL/ventricle) to induce sAD-like pathology. TTR deposition in the choroid plexus and leptomeninges was assessed using immunofluorescence confocal microscopy. Aggregation propensity was evaluated by Thioflavin T staining and native PAGE in brain homogenates with or without exogenous TTR. The influence of TTR on antioxidant enzyme function was tested under native and denaturing conditions using purified catalase and superoxide dismutase. STZ-icv mice showed increased TTR accumulation in leptomeningeal amyloid deposits and upregulated expression in the choroid plexus. These changes were associated with enhanced TTR aggregation. In vitro, TTR sequestration reduced the structural stability and enzymatic activity of antioxidant proteins under stress conditions, suggesting compromised oxidative defence mechanisms. TTR aggregation in the STZ-icv model of sAD may contribute to disease progression by impairing antioxidant defence and increasing oxidative stress vulnerability. These findings highlight the dual role of TTR in neurodegeneration and underscore the need to explore therapeutic strategies aimed at preserving its protective functions in amyloidogenic environments.

DEVELOPMENT OF A NOVEL MULTI-TARGET ANALGESIC CANDIDATE FOR CHRONIC NEUROPATHIC PAIN: PRECLINICAL AND PHASE IA CLINICAL RESULTS

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SZV-1287 (3-(4,5-diphenyl-1,3-oxazol-2-yl)propanal oxime) is a novel and unique multi-target drug, which irreversibly inhibits semicarbazide-sensitive amine oxidase (SSAO) metabolizing primary amines to irritants like methyglyoxal and formaldehyde activating the transient receptor potential ankyrin 1 (TRPA1) and vanilloid 1 (TRPV1) receptors. SZV-1287 also directly antagonizes these non-selective cation channels located predominantly on nociceptive primary sensory neurons, immune cells and several central nervous system regions. Furthermore, its main metabolite, oxaprozin, is a clinically used cyclooxygenase inhibitor, which might have synergistic effect with the mother compound in several pain conditions. We provided proof-of-concept for the ability of single SZV-1287 administration (10-50 mg/kg i.p.) to significantly reduce acute and chronic pain behaviors including the sciatic nerve injury-induced neuropathic hyperalgesia in mice via inhibiting TRPA1 and TRPV1 activation. Repeated 20 mg/kg SZV-1287 injections attenuate chronic arthritis (edema, myeloperoxidase activity, histopathological changes) and related hyperalgesia, L4-L6 spinal dorsal horn neuroinflammation (microgliosis) even in the late phase of the model when neuropathic mechanisms are important. To avoid SZV-1287 conversion to oxaprozin in the stomach, enterosolvent capsule was formulated and tested during preclinical development. SZV-1287 is quickly absorbed, its plasma concentration is stable for 2 h and enters the brain. It does not induce any considerable toxicity either in rodents or in dogs. Phase IA clinical trial (single ascending dosing: 75, 150, 300, 450 mg) has successfully been completed without considerable side effects. If the candidate proves to be safe in humans after repeated dosing for 10 days, it can progress to phase II studies in neuropathic pain patients. Funding: Hungarian Research Network (Chronic Pain Research Group), Pécs, National Brain Research Program 3.0, TKP2021-EGA-16, RRF-2.3.1-21-2022-00015.

MEDITERRANEAN DIET AS A NON-PHARMACOLOGICAL TREATMENT FOR NON-COMMUNICABLE DISEASES

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Mediterranean diet (MD) is one of the most extensively studied dietary patterns worldwide. Based on more than 10,000 published papers in biomedical literature on MD, it stems as one of the healthiest diets, both from the perspective of human and environmental health. Literature on the effects of the MD on health protection, disease prevention, but also on the potential use of MD as a non-pharmacological treatment for non-communicable diseases (NCDs) will be presented, placing special emphasis on cardiovascular health, diabetes and metabolic health, cancer and mental health. Variety, complexity and richness of nutritional components of the MD, including dietary fiber, MUFAs, PUFAs, vitamins, minerals, antioxidants and other phytochemicals could explain why these foods have such beneficial effects on human health and longevity. MD has been shown to have positive effects on gut microbiota composition, its diversity and metabolic activity, possibly playing a crucial role in mediating the effect of MD on various health outcomes. Unfortunately, MD adherence in Mediterranean populations has been in substantial decline, and that was shown in Croatia as well. Even though clinical guidelines for the treatment of NCDs are placing distinct emphasis on lifestyle modification, especially on nutrition and physical activity, they are not commonly put into practice. Hence, we are losing precious potential because MD sustains both human health and preserves environmental biodiversity and sustainability. Additionally, it is part of our culture, tradition and identity, and a driver of healthy local economy, and we should focus on safeguarding it for the next generations of healthy people.

A NOVEL THERAPEUTIC CANDIDATE FOR ALZHEIMER'S DISEASE TARGETING SIGMA-1, SIGMA-2, AND 5-HT2A RECEPTORS AND BUTYRYLCHOLINESTERASE

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Alzheimer's disease (AD) is a progressive neurodegenerative brain disorder with a multifactorial pathophysiology that likely necessitates combination therapies rather than monotherapy. In the AD brain, multiple neurotransmitter systems are disrupted, offering a range of therapeutic targets for prevention and intervention. Sigma-1 (σ1) and sigma-2 (σ2) receptor ligands, serotonin 5-HT2A receptor antagonists, and butyrylcholinesterase (BChE) inhibitors have all shown therapeutic potential in the treatment of AD. We developed a novel multifunctional compound, (R)-(–)-1, which acts as a σ1 and σ2 receptor ligand, a 5-HT2A receptor antagonist, and a BChE inhibitor. Compound (R)-(–)-1 exhibits a high brain-to-plasma ratio in mice and demonstrates significant procognitive effects in two established mouse models of AD: scopolamine-induced amnesia and amyloid-β1–42-induced cognitive impairment. Notably, (R)-(–)-1 does not induce cholinergic side effects, motor impairments, or acute toxicity in mice, supporting its potential as a safe and effective multitarget therapeutic candidate for AD.

MODULATION OF LIPOPOLYSACCHARIDE-INDUCED NEUROINFLAMMATION IN BV2 MICROGLIAL CELLS BY REYNOSIN

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Neuroinflammation is a critical factor in the progression of neurodegenerative diseases, largely mediated by microglial activation. Lipopolysaccharide (LPS) stimulated microglia release pro-inflammatory cytokines and reactive oxygen species, contributing to neuronal damage. Reynosin, a sesquiterpene lactone isolated from the leaves of Laurus nobilis, exhibits anti-inflammatory and antioxidant properties. However, its involvement in microglia-mediated neuroinflammatory processes remains incompletely understood and continues to be a focus of intensive research. BV2-p65 mouse microglial cells, stably expressing an NF-κB luciferase reporter, were treated with reynosin (1, 5, or 10 μM) or vehicle. Inflammatory activation was induced by LPS(1 μg/ml) for 3, 6, or 24 hours. Reynosin was administered as a pre-treatment or post-treatment relative to LPS exposure. Anti-CD36 antibody served as a positive control. NF-κB activation was quantified by luciferase assay and expression of inflammatory markers, iNOS and Iba1, was assessed by western blot. Reynosin alone did not alter NF-κB activity. Pre-treatment with reynosin significantly and dose-dependently suppressed LPS-induced NF-κB activation, with the highest inhibition observed at 10 μM ($p < 0.0001$). Post-treatment with reynosin reduced NF-κB activity, though less effectively. Reynosin significantly decreased LPS-induced iNOS expression at 5 and 10 μM and reduced Iba1 expression at 10 μM. The anti-CD36 antibody robustly suppressed both NF-κB activity and iNOS expression. Reynosin exerts a potent, dose-dependent anti-inflammatory effect in LPS-stimulated BV2 microglial cells, primarily by inhibiting NF-κB activation and reducing expression of iNOS and Iba1. These findings support further investigation of reynosin as a potential neuroprotective agent for neurodegenerative diseases.

TARGETING RNA BINDING PROTEIN SRSF3 AND mRNA TRANSLATION RESTORES IMMUNE RESPONSE IN INJURED BRAIN

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Inflammation is a key component of the innate immune response. Primarily designed to remove noxious agents and limit their detrimental effects, once prolonged and/or inappropriately scaled innate immune response may be detrimental to the host and lead to disease. Microglia are the principal immune cells of the brain. We recently described a novel ribosome-based check-point mechanism involved in translational control of innate immune genes and microglia activation. We used the transgenic line CD11brGFP developed in our laboratory. It expresses FLAG-EGFP RPL10a transgene under the control of the human CD11b promoter. FLAG tag was used for ribosome immunoprecipitation followed by parallel cell specific transcriptome/proteome analysis using the modified translating ribosome affinity purification (EDTA-TRAP) protocol, in the context of different brain injuries. Parallel transcriptome/proteome analysis revealed that after stroke or following LPS challenge highly upregulated immune are not translated resulting in a marked dissociation of mRNA and protein networks in activated microglia/macrophages. The mechanism is based on a selective translational repression of immune mRNAs orchestrated by RNA binding protein SRSF3. Intranasal delivery of siRNA targeting SRSF3 resulted in delayed and controlled enhancement of immunity and was therapeutic in stroke. Our work suggests that targeting SRSF3 and mRNA translation may open new avenues for therapeutic reprogramming of immune response in acute and chronic CNS pathologies.

ARTIFICIAL INTELLIGENCE IN CLINICAL PHARMACOLOGY: FROM DIGITAL ASSISTANT TO PERSONALIZED EDUCATION

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Artificial intelligence (AI) is increasingly influencing clinical pharmacology through its ability to support drug therapy decisions and enhance the education of healthcare professionals. The application of AI tools in both clinical and academic settings opens new possibilities for improving prescribing quality, personalizing learning, and advancing patient care. This presentation examines recent developments in the use of AI in clinical pharmacology, focusing on decision-support systems for prescribing, machine learning models for predicting adverse drug reactions, and intelligent educational platforms. A mixed-methods approach was used, combining a targeted literature review with institutional case examples from both Croatia and international partners. Digital prescribing assistants have demonstrated strong performance in identifying drug interactions and supporting therapy optimization, especially in patients with complex comorbidities. AI-based learning platforms, including adaptive tutorials and personalized feedback systems, have shown improved outcomes in learner engagement, knowledge retention, and clinical reasoning. Pilot projects implemented in academic settings indicate that personalized pharmacology education can be delivered effectively using AI algorithms that adjust content based on individual learner performance. Artificial intelligence has the potential to significantly improve both clinical decision-making and pharmacology education. Its implementation as a digital assistant in prescribing and as a tool for tailored learning represents an important advancement for clinical pharmacologists and medical educators. Ongoing evaluation, quality assurance, and ethical oversight will be essential for its responsible integration into healthcare and academic environments.

PHARMACOGENETICS AND THERAPEUTIC DRUG MONITORING IN PERSONALIZED BRIVARACETAM TREATMENT

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Brivaracetam is a third-generation antiepileptic drug primarily metabolized by hydrolysis, with minor pathways involving CYP2C19 and even CYP2C9. Genetic variability in these enzymes may influence brivaracetam pharmacokinetics. Due to interindividual variability, therapeutic drug monitoring (TDM) is recommended, especially in patients undergoing polytherapy or with altered pharmacokinetics. To assess brivaracetam serum concentrations and their association with CYP2C9 and CYP2C19 genotypes/phenotypes in a real-life clinical setting. This retrospective study included epilepsy patients treated with brivaracetam. TDM was a part of patients' routine clinical management, and clinical study approved by the UHC Zagreb Ethics Committee. Patients were genotyped for CYP2C9*2*3 and CYP2C19*2*17. Brivaracetam concentrations, dose-adjusted concentration (D/C) ratios, clinical data, drug interactions, and genotype/phenotype data were analysed in univariate statistical analysis. TDM data from 100 patients (brivaracetam 50–200 mg/day) were analysed; 70 were genotyped. The median brivaracetam concentration was 6.10 µmol/L (0-21.6 µmol/L). CYP2C19 poor metabolizers (*2/*2) had 55% higher D/C ratios than normal metabolizers (*1/*1; p=0.02); intermediate metabolizers (*1/*2, *2/*17) showed 20% higher exposure (p=0.04); ultra rapid metabolizers (*17/*17) exhibited subtherapeutic concentrations (median: 2.8 µmol/L at 200 mg; p=0.03). CYP2C9 heterozygous carriers (*1/*2, *1/*3) had 22% higher D/C ratios than wild type (*1/*1; p=0.12), with a significant effect in patients on CYP2C9 inhibitors (valproate) (p=0.01). Enzyme inducers (carbamazepine, phenobarbital) reduced brivaracetam concentrations (p<0.01). This small real-life study showed the importance of TDM for brivaracetam and influence of pharmacogenetic variations in CYP2C19, less so in CYP2C9, and drug interactions on brivaracetam concentrations. Further research in larger cohorts is needed.

REVISION OF EU GENERAL PHARMACEUTICALS LEGISLATION

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Based on Pharmaceutical Strategy adopted in November 2021, the European Commission published its "Pharma Package" in April 2023. The aim of the revision of the EU pharmaceutical laws is to ensure that patients across the EU have fair access to safe, effective and affordable medicines, enhance the competitiveness of the EU's pharmaceutical industry, address issues relating to security of supply and mitigate the environmental impact of medicines via better enforcement of environmental rules. The Council of EU agreed its negotiation position (mandate) on June 4, 2025, both for the new Regulation and for the new Directive. The Council's mandates for negotiation introduces several key amendments to the text proposed by the European Commission. Most important are introduction of obligation to supply that gives a Member States the power to oblige marketing authorization holder to make the medicinal product available in sufficient quantities to cover the needs of patients and clarification of the scope of so-called "Bolar exemption" and its expansion to include submissions for procurement tenders. European Parliament adopted its position in April 2024, prior to the Parliamentary elections, and the negotiation of Council and European Parliament can start with a view to reach an agreement on the package.

THE ANALGESIC ACTIVITY OF BOTULINUM TOXIN AND ITS RECOMBINANT DERIVATIVES

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Botulinum toxin type A has been approved for the treatment of chronic migraine. Additionally, it shows promising results in several types of difficult-to-treat chronic pain disorders, such as trigeminal neuralgia and other types of neuropathic pain. Its well-known actions at peripheral cholinergic synapses have led to assumption that its clinical effects on pain are mediated by its action in the peripheral sensory nerve endings. In addition, different recombinant constructs with altered or exchanged membrane acceptor binding moieties, and chimeric neurotoxins combining catalytic domains of different BoNT serotypes, have been designed based on supposed peripheral toxin actions. However, preclinical studies have shown that the BoNT/A-mediate antinociceptive efficacy is not dependent on its peripheral site of action. Moreover, the involvement of peripheral nerve endings was excluded based on observations that BoNT/A action in bilateral and polyneuropathic pain models may be present bilaterally and is dependent on the toxin's axonal transport. Our novel findings suggest that the BoNT/A's antinociceptive activity depends on central SNAP-25 cleavage mediated by toxin transcytosis to second order brainstem/spinal cord synapses. Regarding the efficacy of recombinant toxin molecules, it seems as a general rule that they are substantially less potent than the native BoNT/A. This may translate into higher immunogenicity and potential secondary treatment failure. Further question is whether the recombinant molecules may aim relevant synaptic targets similarly as BoNT/A in the CNS. Thus, a better consensus regarding the relevant targeted synaptic sites/neuronal circuits, that, in turn, may lead to improvement of future development strategies of re-engineered BoNT-based analgesics, is needed. Acknowledgements: The author’s research is funded by Croatian Science Foundation (# UIP-2019-04-8277)

THE SIGMA-1 EUROPE CA23156 COST ACTION

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SIGMA-1 EUROPE (CA23156) is a COST Action of the EU, uniting researchers and experts across Europe to advance the understanding of sigma-1 receptors (S1R), covering all aspects of the sigma-1 receptor pharmacology: medicinal chemistry, pathologies and S1R-based therapies, technological transfer and industrial developments, and knowledge dissemination.

AMYLOVIS-201, A NEW DUAL-TARGET LIGAND ACTING AS AN ANTI-AMYLOIDOGENIC COMPOUND AND AGONIST OF THE σ1 CHAPERONE PROTEIN, IS NEUROPROTECTANT IN ALZHEIMER'S DISEASE MOUSE MODELS

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The aggregation of Amyloid-β (Aβ) peptides is associated with neurodegeneration in Alzheimer's disease (AD). We previously identified novel naphtalene derivatives, including the lead compound Amylovis-201, able to form thermodynamically stable complexes with Aβ species, peptides and fibrils. As the drug showed a chemical scaffold coherent for an effective interaction with the sigma-1 receptor chaperone and as sigma-1 receptor agonists are currently developed as potent neuroprotectants in Alzheimer's disease and other neurodegenerative diseases, we investigated the pharmacological action of Amylovis-201 on the sigma-1 receptor. We report that Amylovis-201 is a potent σ1 agonist by several in silico, in vitro and in vivo assays and that its anti-amnesic and neuroprotective effects involve a pharmacological action at sigma-1 receptors. Furthermore, we show for the first time that sigma-1 receptor acting drugs, such as the prototype agonist PRE-084 or and antagonist NE-100, are able to interact and partially disaggregate Aβ25-35 fibrils. However, Amylovis-201 was the only compound able to fully inhibit Aβ25-35 aggregates formation. Amylovis-201 is therefore a particularly promising dual acting agent, acting as an anti-aggregating agent and neuroprotective sigma-1 receptor agonist that could be highly effective in long-term treatment against neurodegeneration in Alzheimer's disease.

NEW CLINICAL TRIALS REGULATION IN EUROPE

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The implementation of the new Clinical Trials Regulation (EU) No 536/2014 marks a significant shift in how clinical trials are authorized, conducted, and supervised across the EU. Its aim is to harmonize procedures, enhance transparency, and improve safety and efficiency in the clinical trial landscape. This presentation reviews the impact of the Clinical Trials Information System (CTIS), key regulatory milestones since the Regulation became applicable in January 2022, and practical challenges faced by sponsors, investigators, and national competent authorities. It synthesizes data from EMA reports, stakeholder consultations, and early-phase CTIS implementation metrics. Initial implementation shows improved timelines for trial authorizations, with a 20–30% reduction in assessment delays reported in some Member States. CTIS has increased public accessibility of trial data, though user experience challenges and administrative burdens persist. Capacity-building efforts and regulatory support tools have played a critical role in facilitating the transition. The new Regulation represents a major advancement for European clinical research governance. Despite early operational hurdles, it is fostering a more streamlined and transparent environment for clinical trials. Continued stakeholder collaboration will be essential to realize its full potential and maintain Europe's competitiveness in biomedical innovation.

INITIATIVES IN IMPROVING THE AVAILABILITY OF MEDICINAL PRODUCTS

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The European Medicines Agency (EMA) received a reinforced role in crisis preparedness and management for medicinal products and medical devices according to the Regulation (EU) 2022/123 of the European Parliament and Council. Following the aforementioned Regulation, the EMA reinforced new initiatives in improving the availability of medicinal products, among which the implementation of the European Shortages Monitoring Platform (ESMP) in 2025 is highlighted. The ESMP is a centralised platform created for submission, monitoring, prevention and mitigation of shortages of medicinal products throughout the European Union (EU) and the European Economic Area (EEA). Shortage notifications may be submitted to the ESMP for centrally authorised and nationally authorised medicinal products in specific circumstances by the marketing authorisation holder or the national competent authority. Another example of a new initiative in improving the availability of medicinal products in Europe is the launch of the Union list of critical medicines in 2023, which enables and supports the joint work of the EMA, the European Commission (EC) and the Heads of Medicines Agencies (HMA) in taking proactive measures to ensure the supply and availability of medicines at a European level and avoid medicines shortages. The list contains medicinal products for human use whose continued supply is considered a priority in the EU in order to protect and improve the health of its citizens.

NOVEL TREATMENT STRATEGIES FOR GLIA-MEDIATED NEUROINFLAMMATION IN NEURODEGENERATIVE DISEASES: FROM NATURAL EXTRACTS TO TARGETED BIOLOGIC THERAPIES

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This symposium brings together cutting-edge research exploring how glial cells contribute to and can be harnessed to combat neuroinflammation in neurodegenerative conditions. The symposium highlights innovative therapeutic strategies ranging from nanotechnology to natural compounds and advanced biologics. Presentations explore diverse mechanisms and models: functionalized carbon nanotubes demonstrate the potential to modulate astrocyte function and exosomal signaling after traumatic brain injury; psychoplastogens are introduced as fast-acting, multi-target neurotherapeutics capable of regulating glial activity and inflammatory pathways. Human brain organoids offer a next-generation platform for modeling neuroinflammatory responses and testing therapeutic interventions in a human-relevant context. Natural compounds also feature prominently as reynosin, a plant-derived sesquiterpene lactone, significantly suppresses NF-κB signaling and pro-inflammatory markers in activated microglia, suggesting neuroprotective potential. At the molecular level, novel insights into translational regulation by RNA binding protein SRSF3 reveal how modulation of mRNA translation can therapeutically reprogram immune responses in the injured brain. Together, these studies reflect a shift toward integrative, glia-focused strategies that blend molecular precision with translational relevance. The symposium underscores the importance of targeting glial cells not just as contributors to pathology but as key players in neurorepair and regeneration.

DEHYDROEPIANDROSTERONE SULFATE IN ALZHEIMER'S DISEASE: A METABOLOMICS PERSPECTIVE

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Alzheimer's disease (AD) is a complex neurodegenerative condition frequently diagnosed at an advanced stage, with limited treatment options. While the pathological mechanisms primarily impact the central nervous system, they also manifest at periphery. Dehydroepiandrosterone sulfate (DHEAS) is a neurosteroid involved in neuroprotection, synaptic plasticity, oxidative stress and neuroinflammation. Altered DHEAS levels have been reported in several neuropsychiatric disorders, including AD. Metabolomics provides meaningful insights into disease-associated alterations in metabolite profiles. Pharmacometabolomics, which stems from metabolomics, extends this approach by examining how different factors influence individual responses to drug therapy, thereby supporting the advancement of personalized medicine. This study aimed to explore the metabolic profile of AD in patients and in animal model and the effects of DHEAS treatment in animal model. Metabolic profiling in both, human and animal, blood plasma samples, was conducted using the liquid and gas chromatography coupled with mass spectrometry. Human study included 40 AD patients and 40 healthy control subjects. Animal study included 40 male 3xTg mice, grouped as control mice, untreated mice and mice treated with DHEAS. Human study suggested altered lipid, amino acid and energy metabolism with decreased DHEAS abundance in AD. DHEAS treatment in animal model showed a trend toward modulating AD related metabolic disturbances. This study highlights metabolomics as a valuable approach for exploring the biological mechanisms underlying AD pathogenesis and identifying potential treatment strategies. Nevertheless, additional research is required to further elucidate the role of DHEAS in modulating AD pathology.

CIRCULATING EXTRACELLULAR VESICLES AS PREDICTORS OF ANTIDEPRESSANT RESPONSE

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Major depressive disorder (MDD) is a common, highly prevalent, and recurrent mental illness. Currently available therapy for depression involves pharmacotherapy combined with psychotherapy, while dealing with poor response/nonresponse and frequent discontinuation of treatment. The objective of the study is to give better insights into the efficacy and molecular mechanisms behind the effects widely used antidepressant duloxetine and compare this to the mechanism behind the effect of Transcranial Magnetic Stimulation (TMS) in treatment-resistant depression. The study focuses on the potential of circulating extracellular vesicles (EVs) as easily obtainable and non-invasive biomarkers. We aimed to determine metabolic changes in EVs of depressed patients after adequate treatment to explore the potential of pharmacometabolomics in predicting treatment outcome. The gas chromatography-mass spectrometry (GC-MS) was used as an analytical technique for metabolomic profiling. The results on the therapeutic effects of duloxetine and TMS suggest that these treatments differentially affect biochemical pathways related to energy metabolism, amino acid metabolism, and lipid metabolism. This leads to the conclusion that specific metabolites could be used as potential biomarkers for determining the treatment efficiency in depression and to predict good or poor response to treatment, as a key step towards the inevitable personalized and effective medicine approach. By comparing differences and similarities in biomarker signatures between different subtypes of depression and response to different treatments that do not seem to have much in common, we can gain a better understanding of the biological mechanisms of depression. This study was supported by Croatian Science Foundation (project no. IPS-2022-02-2497).

REVERSAL OF TYPE 2 DIABETES WITH LIFESTYLE INTERVENTION

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Type 2 diabetes mellitus (T2DM) has long been considered a chronic and progressive disease requiring lifelong pharmacotherapy. However, recent evidence challenges this paradigm, showing that remission is achievable through lifestyle changes and appropriate deprescription. This presentation critically examines key clinical trials (DiRECT, DIADEM-I, CORDIOPREV) and the physiological basis behind T2DM remission, including hepatic and pancreatic fat reduction and β-cell recovery. Additionally, it explores the role of deprescribing in diabetes care—emphasizing reduction of overtreatment, improvement in quality of life, and medication safety. Lifestyle interventions involving significant caloric restriction, structured weight loss, and Mediterranean or low-carbohydrate diets have resulted in sustained diabetes remission in up to 50% of selected patients. Deprescribing antidiabetic medications, including insulin, has been safely implemented under clinical supervision. Remission has been associated with improved insulin sensitivity, reduced medication burden, and potential reversal of β-cell dysfunction. Reversal of T2DM via lifestyle intervention is an achievable goal in motivated patients, especially early in the disease course. A paradigm shift is needed—from a glucose-centered, pharmacological model to a broader, patient-centered approach focused on modifying the underlying disease process. Intensive lifestyle change has the potential not only to prevent and better manage, but also to reverse chronic non-communicable diseases such as type 2 diabetes. Early education, shared decision-making, and interdisciplinary support are essential for sustaining remission and minimizing overtreatment. Every patient has the right to know that remission is possible, and to receive the necessary support in pursuing it.

PHARMACOKINETICS AND PHARMACODYNAMICS OF INTRANASALLY ADMINISTERED INSULIN IN A RAT BRAIN

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Understanding of the insulin mediated effects on signalling and metabolism is important not only for fundamental knowledge of insulin's action in the brain, but also for elucidating the mechanism of therapeutic potential in neurodegenerative disorders with underlying brain metabolic dysfunction. One of the main goals of this research is to clarify and explore the time-dependent regular insulin distribution and activity in the rat brain and to compare it to the central distribution of FITC-labelled insulin following intranasal administration. Male Wistar rats were administered 2 IU of FITC-labelled or regular insulin intranasally and sacrificed 3, 7.5, 15, 30, 60 and 120 min after. Control animals were sacrificed without intranasal administration. Insulin, C-peptide and glucose concentration were measured in plasma and CSF, while levels and activity of insulin signal transduction network were measured in the brain and epithelia. Regular insulin rapidly distributed to all brain regions following intranasal administration and was quickly utilized and/or metabolized. FITC-insulin also distributed to all brain regions but in lower concentrations and was not immediately utilized and/or metabolized. Intranasal insulin positively influences insulin secretion seen as increment of C-peptide and insulin in the periphery and in distinctive brain regions. Secondary activation of AMP-activated protein kinase and calcium/calmodulin-dependent protein kinase could be due to brain region-dependent negative feedback mechanism on the overstimulated insulin signalling pathway. A significant difference in the distribution and metabolism of regular versus FITC-labelled insulin was observed, raising concerns about the use of labelled agents in pharmacokinetic studies. The insulin dose was likely too high, leading to its transport back to epithelia through unknown mechanisms, which could be of relevance for human dose reduction. Possible beneficial insulin action could be due to overstimulation of the insulin-signalling pathway with subsequent inactivation through insulin receptor substrate phosphorylation at Ser307. Acknowledgments: This work was supported by the Croatian Science Foundation under the project number HRZZ-2022-10-1895 and University of Zagreb project (10106-23-2516). The work of doctoral student Antonia Krsnik has been fully supported by the "Young researchers' career development project – training of doctoral students" of the Croatian Science Foundation (DOK-NPOO-2023-10-3354).

NEW DRUGS IN NEURODEGENERATIVE DISEASES

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Neurodegenerative diseases such as Alzheimer's disease and Down syndrome–associated dementia represent an urgent and growing challenge in medicine. A common feature across these conditions is the presence of metabolic dysfunction in the brain, including altered glucose metabolism, mitochondrial impairment, and oxidative stress, which contribute significantly to neuronal loss and cognitive decline. Recent advances in understanding these metabolic derangements have opened new opportunities for therapeutic intervention. This symposium will focus on the development of novel drugs that specifically target brain metabolism as a strategy to modify the course of neurodegenerative disease. Special emphasis will be placed on disorders such as Alzheimer's disease and Down syndrome, where metabolic biomarkers precede clinical symptoms. In addition, transthyretin amyloidosis, a condition with both neurodegenerative and systemic manifestations, will be highlighted for its unique metabolic and pathology changes in the brain, and the therapeutic potential of stabilizing transthyretin with small molecules will be discussed. We will explore agents designed to enhance mitochondrial function, restore cerebral glucose utilization, reduce oxidative damage, and modulate neuroinflammation and increase cholinergic transmission. The importance of developing drugs with multiple mechanisms of action—targeting interconnected pathways—is increasingly recognized as essential for addressing the complexity of these diseases. By integrating insights from molecular metabolism with innovative drug development, this session aims to present a comprehensive view of how targeting brain energy homeostasis can yield effective disease-modifying therapies.

TEACHING PHARMACOLOGY IN SCHOOL OF DENTAL MEDICINE

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Although the necessity of basic medical sciences in dental education is well-known, basic science courses strongly differ from the clinical courses in contextual, structural and executive manner, leading most of students to strongly prefer dental clinical courses. The aim of this study was to follow the interest of dental students in pharmacology after the implementation of different teaching methods in mandatory and elective pharmacology courses of dental curriculum. Within 10-year period, 2015 -2025, teachers from the Department of Pharmacology, intervened in restructuring of mandatory 3rd year Pharmacology course and three elective courses related to pharmacology (4th year Toothpaste preparation, 5th year Clinical pharmacology and 6th year Medication prescribing in clinical dental practice) with different teaching methods. Followed outcomes were number of enrolled students, student's grades, student's surveys comments on courses, teacher's grades received from student's surveys and course's rank compared to all other courses in curriculum (including clinical courses). Number of students enrolled in pharmacology elective courses significantly increases during 10-year period, for all years of study (4th, 5th and 6th). Average grade in mandatory pharmacology courses in 3rd year significantly improved. Scored teacher's grades and course's ranks resulted in 6 Dean's awards for best teacher / best course. Intervention in teaching pharmacology for dental medicine students including case-based problem solving, problem-based learning, flipped classroom and digital media tools significantly improved student's interest in pharmacology as well as student's learning outcomes.

MODULATION OF REPETITIVE BEHAVIOURS IN AUTISM: TRANSLATIONAL NEUROIMAGING AND PHARMACOLOGICAL INSIGHTS

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Autism spectrum disorder (ASD) is a complex neurodevelopmental condition marked by repetitive behaviours and social communication difficulties. A disruption in the balance between excitatory and inhibitory signalling has been suggested as a core feature of ASD pathophysiology, yet effective pharmacological treatments remain limited. N-acetylcysteine (NAC)—which modulates glutamatergic transmission via activation of the cystine/glutamate antiporter—and oxytocin have shown promise in alleviating core symptoms, particularly repetitive behaviours, in autistic individuals. The BTBR mouse model exhibits robust ASD-like behaviours, including pronounced stereotypies. Here, we investigated the acute effects of NAC and oxytocin on behavioural and neural outcomes in BTBR and control (C57BL/6J) mice. Using a combination of translational neuroimaging (i.e. perfusion-based functional MRI) and behavioural assessments, we examined the acute effects of NAC and oxytocin on repetitive behaviours and neural activity in BTBR and C57BL/6J control mice. By integrating translational neuroimaging with behavioural assessments, we identified key neural substrates associated with distinct forms of repetitive behaviours in the BTBR mouse model of autism, as well as mechanisms through which NAC and oxytocin alleviate these phenotypes. Our findings underscore the value of fMRI in delineating large-scale neural circuitry implicated in neuropsychiatric disorders and in evaluating the efficacy of pharmacological interventions in preclinical models.

FUNCTIONALIZED CARBON NANOTUBES MODIFY ASTROCYTE FUNCTION AND MODULATE EXOSOMAL miRNA PROFILES FOLLOWING IN VITRO TRAUMATIC BRAIN INJURY

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The integration of nanomaterials into neurotherapeutics is emerging as a transformative strategy for treating traumatic brain injury (TBI). Carbon-based nanomaterials such as single-walled carbon nanotubes (SWCNTs) exhibit unique physicochemical properties suitable for CNS repair. Astrocytes, as principal regulators of neurotransmitter homeostasis and inflammatory signaling, are attractive targets for modulating secondary injury responses post-TBI. We utilized an in vitro model of severe TBI by applying mechanical stretch injury to primary mouse astrocyte cultures. Cells were treated with SWCNTs conjugated with polyethylene glycol (SWCNT-PEG). Cell viability, oxidative stress, and expression of glutamate transporter EAAT1 were assessed post-treatment. Cytokine secretion was quantified via cytokine array kit. Exosomes were isolated from conditioned media, and exosomal miRNAs (miR-16, miR-21, miR-26, and miR-146) were quantified using quantitative real-time PCR (qRT-PCR). SWCNT-PEG treatment did not induce additional cytotoxicity or oxidative stress in stretch-injured astrocytes. Notably, SWCNT-PEG mitigated injury-induced downregulation of EAAT1. Cytokine profiling revealed a significant upregulation in the secretion of a number of cytokines, indicating a shift toward an anti-inflammatory and neuroprotective secretome. qRT-PCR of exosomal RNA revealed variation in levels of miRNAs, associated with in vitro TBI and SWCNTs treatments. Our results demonstrate that SWCNT-PEG enhance critical astrocyte functions following traumatic injury by preserving glutamate transporter expression, promoting a beneficial cytokine miRNA profile, and influencing astrocyte communication through modulation of exosomal miRNA cargo. These findings support the therapeutic potential of functionalized carbon nanotubes and suggest a novel role for nanomaterial-based interventions in modulating astrocyte-mediated repair mechanisms in TBI.

BIOMARKERS OF ALZHEIMER'S DISEASE

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Dementia is an umbrella term that encompasses various types of dementia including the most frequent type, Alzheimer's disease (AD). Besides AD, common subtypes are vascular, Lewy bodies, frontotemporal, mixed and young-onset dementia. Dementia results from the organic disease of the brain and is characterized with a myriad of neurological or psychiatric symptoms, leading to progressive or persistent loss of intellectual functioning, impairment of memory and abstract thinking, personality change, that gradually worsen over time. AD diagnosis includes clinical evaluation, physical, neurological and neuropsychological tests, and biomarkers such as MR imaging, CT, PET (FDG, amyloid and tau PET), cerebrospinal fluid and blood brain biomarkers. Compared to other biomarkers, blood-based biomarkers are easy to access, less invasive, scalable, they save time and reduce costs. They are divided into those associated with: 1) amyloid β peptides (Aβ42-to-Aβ40 ratio) and phosphorylated tau (p-tau), 2) neuronal loss, neurodegeneration, or synaptic degeneration, and 3) neuroinflammation mediated by glial cells. The most important characteristic of biomarker is to predict the increased risk/hazard for development of AD with high sensitivity and specificity. Novel data (Grande et al., Nat Med 31, 2027-2035, 2025) revealed that elevated baseline concentrations of p-tau181, p-tau217, neurofilament light chain, and glial fibrillary acidic protein were associated with a significantly increased AD/dementia risk and had the strongest predictive values. Other frequently assessed blood-based biomarker, Aβ42-to-Aβ40 ratio, showed a weak association. These data emphasize that combination of biomarkers and not a single marker determination should be used to screen the risk for AD/dementia.

ENVIRONMENTAL RISK ASSESSMENT OF MEDICINAL PRODUCTS FOR HUMAN USE

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The advancement of medicine and pharmacy has led to the discovery of new diseases and therapies. Increasing population, longer life expectancy, and broader access to medications have contributed to a rise in pharmaceutical consumption. Since the 1990s, pharmaceuticals have been identified as environmental contaminants, with numerous active substances detected in various environmental compartments, including wastewater, surface and groundwater, soil, and air. Environmental Risk Assessment (ERA) is a mandatory requirement for authorisation of medicines in the EU. Overview of the environmental presence of pharmaceuticals and outline of the methodology of ERA for the purpose of authorisation of medicines is provided. The structure of ERA, relevant ecotoxicological endpoints, and the use of environmental fate data in risk characterization is discussed. An overview of current EU legislation and guidelines on ERA is presented. Findings from ERA can trigger national and EU-level risk management actions, such as implementation of risk mitigation measures, inclusion in environmental monitoring programs, and consideration for regulatory follow-up. Moreover, certain pharmaceuticals identified through ERA and environmental monitoring have been proposed for or included in the EU Water Framework Directive watchlist, indicating their relevance for broader environmental policy. Thus, ERA not only supports regulatory decision-making for medicines but also serves as an early warning mechanism for substances of emerging environmental concern. Pharmaceuticals in the environment represent a growing global concern. ERA is a crucial tool for identifying and managing risks to environmental health. Strengthening regulatory requirements and improving environmental monitoring can help minimize pharmaceutical pollution and protect ecosystems.

PHARMACOLOGICAL TREATMENT OF ALZHEIMER DISEASE FROM THE CLINICIAN PERSPECTIVE

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Alzheimer disease (AD) is an important health-care challenge. Literature search was conducted to summarize the ongoing recommendations for pharmacological treatment of AD. Two main treatment targets are cognitive dysfunction and behavioral disturbance. Standard treatments are acetylcholinesterase inhibitors (ACE-I) and memantine. They provide moderate but meaningful benefits in postponing cognitive decline. Recently introduced anti-amyloid antibodies represent the paradigm shift. Aducanumab, lecanemab and donanemab are approved by FDA, and lecanemab by EMA. Without treatment, dementia would progress from mild to severe stage in about three years. With ACE-I this worsening could be postponed by several years. Anti-amyloid therapy may have the potential of both such decline and improving the existing deficit. Current evidence supports their use only in mild stages, and the data regarding long-term efficacy and safety are lacking. Newer-generation antipsychotics are widely prescribed in the treatment of psychosis, agitation and aggression. There is evidence of efficacy for olanzapine, risperidone, brexpiprazole, aripiprazole and quetiapine from meta-analyses, amisulpride from randomized trial, clozapine from case reports and no data so far for cariprazine, lurasidone and paliperidone. Their efficacy against placebo was usually small or moderate, and associated with potential harm. Antipsychotics are given in minimal doses and are usually discontinued after cessation of symptoms. Serotonin reuptake inhibitors were effective in agitation, and methylphenidate in patients with apathy. No evidence supports the use of benzodiazepines. Clinical decisions are individualized due to complex nature of the disease, differences in symptoms and high number of comorbid conditions.

SPINAL LEVEL-SPECIFIC ARCHITECTURE OF THE MOUSE LIGAMENTUM DENTICULATUM

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Coordinated vertebral-column movements in vertebrates transmit phasic tension changes directly to the spinal cord via the denticulate ligament. Although in fish, reptiles and amphibians such intraspinal stretch has been linked to specialized sensory systems, less is known about how these tension changes affect the ligamentum denticulatum in limbed mammals. Yet scarce in research, human, porcine and canine specimens already reveal species-specific differences. Here, we performed a comprehensive histoanatomical characterisation of the ligamentum denticulatum in mouse, with samples taken from the cervical, thoracic, lumbar and sacral levels of the spinal cord. Juvenile wild-type mice were euthanized and their spinal cords dissected both with and without surrounding vertebrae. Samples retaining vertebrae were decalcified in 5 % EDTA over a 2-week period to soften the bone for sectioning. Tissue was embedded in 2 % agarose and mounted for cutting on a high-speed vibratome, on coronal sections of 40, 80, and 100 µm thickness. Sections were stained with Masson's trichrome to visualize collagen fibers and separate sections underwent immunohistochemistry for collagen type I and NeuN staining. We quantified fiber thickness, collagen ratio and fiber orientation using confocal microscopy and images were processed using FIJI. The histoanatomical characterization revealed level specific differences in collagen fiber thickness and ligament distribution in different levels of spinal cord which could correspond to the differing tension demands required for movement at each level. The specific histoanatomical differentiation of the ligamentum denticulatum in mouse could suggests a more complex role, extending beyond mechanical anchoring.

PHARMACOGENETICS GUIDED DRUG THERAPY: INTEGRATING PATIENT SPECIFIC SAFETY FACTORS IN A HOLISTIC APPROACH TO PERSONALIZED MEDICINE

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Polypharmacy in the elderly is associated with an elevated risk of adverse drug reactions (ADRs), particularly due to drug-drug interactions (DDIs) and drug-gene interactions (DGIs). Individual variability in drug metabolism caused by genetic polymorphisms and non-genetic factors can significantly alter therapeutic outcomes. We aimed to investigate pharmacogenetics in a holistic approach and see if this can improve drug safety in older patients with complex medication regimens. We analyzed pharmacokinetic and genotyping data from multiple clinical and real-world cohorts in a meta-analytic approach and in primary datasets from TDM, focusing on CYP2D6 and CYP2C19 enzyme activity. Pharmacogenetic based predictions of individual drug clearance was done for different substrates of these enzymes, and in primary TDM data, other influences on drug clearance were studied in addition to pharmacogenetics. Drug concentration data and medication profiles were used to model phenoconversion and predict drug-drug-gene interactions. Our data confirmed significant variability in drug metabolism linked to CYP2D6 and CYP2C19 genotypes. However, comedications frequently caused phenoconversion, overriding genotype-predicted activity. Solanidine-based phenotyping showed strong concordance with metoprolol, indicating that dietary markers may be useful as probe drugs for phenotyping, especially in geriatric patients. In addition to geno- and phenotypes, we observed a clear age-dependent increase in plasma drug concentrations for antidepressants, leading to concentration doubling by age 80. Combining pharmacogenetic profiling with phenotyping and real-world clinical data enables more accurate prediction of metabolic capacity and drug interactions in polypharmacy. Such an integrated approach supports safer and more effective personalized pharmacotherapy in aging populations and highlights the importance of age- and context-specific drug safety assessments in clinical pharmacology practice in order to avoid severe consequences in elderly people, such as falls in frail patients.

NOVEL RESEARCH APPROACHES FOR PREVENTION AND THERAPY OF MENTAL DISORDERS

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Mental disorders encompassing a wide range of conditions, from neurodevelopmental to neurodegenerative diseases, are characterized by significant disturbances in individual's cognition, emotions or behavior. Complex multifactorial basis of mental illnesses, such as depression, dementia, or autism spectrum disorder, requires an integrative approach including both prevention and treatment strategies. Animal models offer insights into neurobiological and behavioral aspects that cannot be studied in humans due to inaccessibility of the brain or ethical and financial barriers to clinical testing. Preclinical neuroimaging in combination with behavioral assessments offers a powerful, non-invasive, and translatable tool in identifying key neural substrates and mechanisms of mental disorders, as well as evaluating the efficacy of pharmacological interventions. One novel strategy for enhancing the translational impact of preclinical neuroimaging is pharmacological fingerprinting of neuroactive drugs that may accelerate and refine the drug development process for mental illnesses. Metabolomics, a comprehensive analysis of metabolites within a biological system, is an emerging omics technology that also holds promise for providing meaningful insights into underlaying mechanisms, identifying biomarkers, and developing new personalized therapies. Pharmacometabolomics, which stems from metabolomics, uses an individual's metabolic profile to predict or evaluate their response to drug treatment, enabling precision medicine. Metabolomic changes in circulating extracellular vesicles, the important mediators of intercellular communication, represent potential easily obtainable and non-invasive biomarkers of mental diseases, their progression and therapy response monitoring. These advances in the brain research aim to strengthen the bridge from bench to bedside, as well as to promote tailored, personalized approach to prevention and therapy.

THE ROLE OF THE MICROBIOTA IN NEURODEGENERATION

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The gut microbiota represents a complex ecosystem that plays a crucial role in maintaining host health, including the functioning of the central nervous system (CNS). A bidirectional communication pathway, commonly referred to as the gut–brain axis, enables the microbiota to influence brain function through neuroendocrine, metabolic, and immunological mechanisms. Growing evidence suggests that an imbalance in the composition of the gut microbiota, known as dysbiosis, contributes to the development of several neurodegenerative disorders, such as Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis. The microbiota affects the CNS through several key mechanisms: a) by activating microglia, which, under dysbiotic conditions, acquire pathological features associated with inflammation and neurodegeneration; b) by modulating the activity of astrocytes and oligodendrocytes, further promoting neuroinflammation and myelin degradation; c) through the production of microbial metabolites such as short-chain fatty acids (SCFAs), which can exert both protective and detrimental effects depending on the context; d) by synthesizing neurotransmitters and hormones (e.g., GABA and serotonin), thereby directly influencing neurological functions. Recent studies indicate that modifying the gut microbiota—whether via fecal microbiota transplantation (FMT), or the use of prebiotics and probiotics—may offer therapeutic benefits. However, the outcomes of such interventions vary depending on multiple factors. For instance, SCFAs may reduce inflammation and support neuronal health in some conditions, while in others, they may exhibit pro-inflammatory properties that exacerbate CNS damage. Additionally, sex-specific differences play a role in modulating the microbiota–CNS axis. While further research is needed to fully understand these complex interactions, current findings provide a promising foundation for the development of microbiota-targeted strategies in the prevention and treatment of neurodegenerative diseases.

AMENDED VARIATION REGULATION – OVERVIEW AND PRACTICAL EXPERIENCES

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Variations are governed by specific regulation and guidelines. Amended variation regulation revised the current rules, setting out the procedures for post-authorisation changes to marketing authorisation, to make the lifecycle management of medicinal products more efficient. Commission Regulation (EC) No 1234/2008 of 24 November 2008 concerning the examination of variations to the terms of marketing authorisations for medicinal products for human use, has been amended few times since it is in force, but the most important amendment entered into force at the beginning of 2025 with Commission Delegated Regulation (EU) 2024/1701 of 11 March 2024. Key amendments of new regulation: 1) Article 7a: Super-grouping of type IA variations across multiple marketing authorisations held by the same holder now can include nationally authorised medicinal products; 2) Article 8: When a minor variation type IA is made, the holder shall submit simultaneously to all relevant authorities a notification within 12 months following the implementation of the variation as an annual update for all minor variations of type IA: 3) Article 20: Mandatory work-sharing procedures for variations involving the same marketing authorisation holder for variations type IB and type II. This approach aims to avoid duplication of efforts by national competent authorities and streamline the evaluation process. These amendments aim to create a more flexible and efficient regulatory framework for the lifecycle management of medicinal products, ensuring that the procedures for post-authorisation changes are better aligned with current scientific and technological developments.

BRAIN INSULIN RESISTANCE AS A HALLMARK OF ACCELERATED AGING IN DOWN SYNDROME

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Brain insulin resistance (bIR) is a key driver of Alzheimer's disease (AD) with aging. Down syndrome (DS), characterized by accelerated aging, is a genetic for of AD, and offers a unique model to explore age-related neurodegenerative mechanisms. Building on our previous findings of bIR in children with DS (under 15 years), we sought to determine whether these alterations persist into adulthood and contribute to AD in DS. Neuronal-drived extracellular vesicles (nEVs), as "liquid biopsies," offer a minimally invasive platform to assess brain health. nEVs were isolated from plasma samples of sex- and age-matched DS individuals (20-40 y, n=35), AD (60-78 y, n=30) and age-matched controls (DS: n=30; AD: n=25). nEVs were chracterized according MISEV guidelines. Levels of bIR markers were measured through a multiplex technology, while circulating AD biomarkers by SIMOA. Our results show higher levels of bIR markers in nEVs from DS vs controls. Circulating AD markers are in the pseudonormality ranges previously measured for DS. Multivariate analyses show a signifcant age-related accumulation of bIR markers, independently of AD pathology. Comparable levels of bIR markers between DS and AD subjects were observed. Our findings confirm the early-onset of bIR in DS individuals independently of AD-like neuropathology posing bIR among the main drivers of neurodegeneration in DS. This reinforces the concept of DS as a model of accelerated aging, where early impairments in insulin signaling may set the stage for increased neuronal vulnerability and AD.

POSTER SEKCIJE / POSTER SESSIONS

METABOLIC PROFILING OF MAJOR DEPRESSIVE DISORDER AND TREATMENT-RESISTANT DEPRESSION: UNTARGETED METABOLOMICS ANALYSIS OF PLASMA SAMPLES

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Major depressive disorder (MDD) is a prevalent psychiatric illness that profoundly affects daily functioning. Despite numerous pharmacological and non-pharmacological interventions, around 30% of patients exhibit inadequate responses, resulting in treatment-resistant depression (TRD). TRD represents a significant clinical challenge due to its association with increased chronic morbidity and suicide risk. This highlights the urgent need for reliable biomarkers to guide personalized treatment. Monitoring metabolic alterations pre- and post-treatment enables a clearer differentiation between MDD and TRD. This study conducted untargeted metabolomic profiling of plasma in patients with MDD and TRD prior to and following an 8-week treatment with duloxetine (N=33), bright light therapy (BLT, N=31), esketamine (ESK, N=29), or transcranial magnetic stimulation (TMS, N=24). A cohort of healthy controls (N=36) was also included. Plasma samples underwent deproteinization and derivatization, then were analyzed with an Agilent 7890A gas chromatograph linked to a 5975C quadrupole mass spectrometer. Metabolites were identified utilizing NIST and Fiehn libraries. At baseline, TRD patients showed reduced levels of arachidonic acid (AA), glycine, and phosphoric acid (PA) compared to MDD and healthy controls. After treatment, duloxetine increased AA in MDD, while BLT elevated both AA and PA in TRD. Esketamine reduced hydroxyisobutyric acid, acetoacetate, and uric acid, and increased AA. TMS led to decreased fatty acyls, serine, acetoacetate, and PA. These findings highlight distinct metabolic patterns differentiating MDD from TRD and suggest potential indicators for assessing therapeutic response. However, further research is required to confirm these findings. This study was funded by the Croatian Science Foundation (IPS-2022-02-2497).

THE INFLUENCE OF ADAPTATION TO RED WINE CONSUMPTION ON FEEDING BEHAVIOR, BODY MASS, AND SURVIVAL FROM MYOCARDIAL INFARCTION IN RATS

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Red wines are known for its cardioprotective effects, largely due to being rich with bioactive compounds. However, their higher tannin content can reduce palatability compared to lighter wines. This study first investigated how different red wine consumption adaptation regimes influenced feeding behaviour and final body mass of rats compared to water only controls. It also assessed the effects of moderate red wine consumption on survival after myocardial infarction (MI). Male Sprague Dawley rats (n = 47) were randomized into five groups: water-only control, three red wine (RW) groups with 2-, 4-, or 6-day adaptation periods, and one RW group without adaptation. After four weeks of drinking period, MI was induced by ligation of the left anterior descending artery. Survival was higher in RW groups (86.7%) than controls (61.5%), with no significant differences among RW groups based on adaptation. Wine, water and food consumption and weight gain showed no significant differences across RW consuming groups. However, total fluid and food intake, energy intake, and final body weight differed significantly between the control and RW drinking animals. RW consumption was not significantly different when animals that survived MI were compared with animals that did not survive. Moderate RW consumption improves survival after MI. Adaptation to RW seemed to not be required, as no differences were found between red wine consuming groups. Changes in feeding habits and body weight likely reflect metabolic effects of RW constituents on gastrointestinal and neurological systems.

SELECTIVE IMMUNOMODULATORY EFFECT OF EUCALYPTOL (1,8-CINEOLE) ON BV2 MICROGLIA

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Eucalyptol (1,8-cineole) is a naturally occurring monoterpene widely used in the treatment of chronic inflammatory airway diseases, due to its well-documented anti-inflammatory and antioxidant properties. In the central nervous system, microglia are the resident innate immune cells responsible for debris clearance, tissue repair, and maintenance of neural homeostasis. In neurodegenerative conditions, microglia often shift toward a persistently activated pro-inflammatory (M1) phenotype, exacerbating neuronal injury. Recent evidence suggests that eucalyptol may exert neuroprotective effects by modulating microglial activation states and enhancing anti-inflammatory signalling pathways. Murine BV2 microglial cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum, 1% antibiotic–antimycotic solution, 1% L-glutamine, and 1% HEPES buffer. At 70–80% confluency, cells were stimulated with 1 µg/mL lipopolysaccharide (LPS) to induce a pro-inflammatory phenotype. After 24 hours, cells were treated with 5 µM eucalyptol for 24 hours. Subsequently, cells were collected for Western blot and immunofluorescence analysis to assess phenotype-specific marker expression. At the concentration of 5 µM eucalyptol showed no cytotoxicity in BV2 cells. As expected, LPS reduced CD206 expression, a marker of the anti-inflammatory (M2) phenotype. Eucalyptol did not reverse this reduction. However, a pro-inflammatory (M1) marker was significantly attenuated by eucalyptol. Eucalyptol is non-toxic to BV2 microglia and selectively suppresses M1-associated markers. However, it does not promote a shift toward the anti-inflammatory M2 phenotype under the tested conditions.

EFFECTS OF ANTIDEPRESSANT FLUOXETINE IN THE PRESENCE OF INCREASED COPPER LEVELS

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Pharmacological treatments for depression primarily target altered monoamine neurotransmission. However, approximately 30% of patients do not respond to standard drug interventions. Various factors, including oxidative stress (OS) and elevated cortisol levels, have also been implicated in disease development. Transition metals have been recognized as important contributors to OS and depressed patients often exhibit elevated copper levels. However, role of copper in the development of depression, as well as its potential impact on treatment response, remains insufficiently explored. We investigated effects of fluoxetine, a commonly prescribed antidepressant, in neuroblastoma SH-SY5Y cells treated concomitantly with cortisol and two different concentrations of copper (low and high) for 48 hours. We studied effects of fluoxetine on viability (MTT test, ATP levels, protease activity), OS parameters (ROS levels, GSH/GSSG ratio), cell death (lactate dehydrogenase activity, caspase-3/7 activity, staining with Hoechst 33342 and propidium iodide) and expression of genes involved in copper regulation and antioxidant protection. In cells exposed to high concentrations of copper, fluoxetine exacerbated toxic effects of combined cortisol and copper treatment, demonstrating prooxidative and proapoptotic properties. These findings suggest that copper levels and OS severity may affect cellular effects of fluoxetine. The results of the research could contribute to better understanding of the mechanisms that may influence the effectiveness of pharmacotherapy and help in the development of adjuvant antioxidative interventions that potentially could improve treatment outcomes.

PHARMACOGENOMICS OF ATORVASTATIN – THE ROLE ABCG2, CYP3A4, CYP3A5, AND SLCO1B1 VARIANTS

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ABCG2 c.421C>A and SLCO1B1 c.521T>C variants are associated with reduced transporter function and higher statin exposure, and SLCO1B1 increased function variants with lower exposure to some statins. This study analysed associations between ABCG2, CYP3A4, CYP3A5, and SLCO1B1 variants and phenotypes with atorvastatin adverse drug reactions (ADRs). This case-control study included atorvastatin-treated patients. Cases were patients experiencing myotoxicity, hepatotoxicity, and other atorvastatin-related ADRs; controls were subjects free of ADRs. Genotyping of ABCG2 c.421C>A, SLCO1B1 c.388A>G, c.463C>A, c.521T>C, c.1929A>C, CYP3A4*22, and CYP3A5*3 by TaqMan real-time PCR. Clinical and laboratory data, ADRs, and potential drug–drug interactions were assessed. The analysis included 606 subjects (208 cases, 298 controls), cardiovascular and metabolic disease patients, from the larger PGx CardioDrug cohort (n=1908). ADRs included myotoxicity (16.4%), hepatotoxicity (7%), other ADRs (9%), and statin therapy switching (20.8%). SLCO1B1 c.521T>C carriers had higher odds (OR=1.67, 95% CI: 0.99-2.84) of developing myotoxicity compared to non-carriers. CYP3A4*22 carriers had higher odds (OR=2.1, 95% CI: 1.04-4.33) of statin therapy switching compared to normal metabolisers. SLCO1B1 increased/highly increased function phenotypes (vs. poor) were associated with 30% probability of ADRs. ABCG2 c.421C>A was not associated with ADR risks. Preliminary results suggest CYP3A4*22 may be linked to increased statin therapy switching. SLCO1B1 c.521T>C is associated with a higher risk of atorvastatin related myotoxicity, and SLCO1B1 increased/highly increased function phenotypes with a lower risk of developing atorvastatin related ADRs. Acknowledgements: Supported by the “Pharmacogenomics in prediction of cardiovascular drugs adverse reaction – PGx CardioDrug” project, funded by the Croatian Science Foundation.

INTRANASAL INSULIN MODULATES INSULIN RECEPTOR SIGNALING IN THE RAT BRAIN: A REGION-, TIME-, AND DOSE-DEPENDENT ANALYSIS

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Metabolic dysfunction and brain insulin resistance are features of Alzheimer's disease (AD), characterized by impaired insulin signalling despite adequate systemic insulin level. Proper activation of the insulin receptor (IR) and its downstream target, insulin receptor substrate (IRS), is essential for neuronal glucose uptake. IRS activation is marked by tyrosine (Tyr) phosphorylation, while serine (Ser) phosphorylation indicates inhibition. Clinical research has shown that intranasal (IN) insulin improves cognition in AD without peripheral side effects, and its effect on insulin signaling is of particular interest. This study compared the effects of acute IN insulin (0.5, 2, and 6 IU) on IRS activity in the hypothalamus (HPT), hippocampus (HPC), and temporal cortex (TC) of male Wistar rats. Animals were sacrificed at 3, 7.5, 15- and 30-minutes post-administration (n=6/group). IRS activity was assessed by Western blot as the phospho/total protein ratio. Phosphorylation of IRSSer307 increased over time in the HPC and TC after 0.5 and 2 IU of intranasal insulin, while 6 IU induced early IRS inhibition that subsequently declined in HPC. In the HPT, only 0.5 IU caused a notable increase in Ser307 phosphorylation. IRSTyr608 phosphorylation in HPC was rapid and sustained after 0.5 IU, and transient after 2 IU. In TC, activation increased with all doses, while HPT showed minimal activation changes across treatments. These findings suggest that intranasal insulin modulates IRS activity in a dose- and region-specific manner. Low and moderate doses enhanced IRS activation in HPC and TC, while high doses induced early inhibition. HPT responses were limited, indicating potential regional insulin sensitivity differences. Acknowledgments: This work was supported by the Croatian Science Foundation under the project number HRZZ-2022-10-1895 and University of Zagreb project (10106-23-2516). The work of doctoral student Antonia Krsnik has been fully supported by the "Young researchers' career development project – training of doctoral students" of the Croatian Science Foundation (DOK-NPOO-2023-10-3354).

SIGNIFICANCE OF INTEGRATED OPTICAL CELLS DENSITY FOR EVALUATION OF PHARMACOTHERAPEUTIC RESPONSE TO MONOCISPLATIN IN LOCALLY ADVANCED CERVICAL CANCER

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The gold standard for the locally advanced cervical cancer (ACC) treatment is monocisplatin chemotherapy (MC). The aim of this work was to examine the ACC pharmacotherapeutic response (PR) to MC depending on the Ki-67 index (KI) and integrated optical cells density (IOCD). The research was a prospective study – 59 ACC patients treated at the Oncology Clinic University Clinical Center Nis, Serbia which were divided into three groups based on the KI value tertile. All subjects received chemotherapy - MC with dose of 40 mg/m2 in six weekly cycles. The PR to the MC in relation to the KI and IOCD was monitored one year after the start of the first chemotherapy. A statistically significant increase (SSI) in the IOCD was demonstrated in subjects with KI > 60% compared to the group with KI < 40% for $p < 0.001$, as well as in subjects with KI between 40 and 60 compared to the group with KI < 40% for $p < 0.05$. SSI in the IOCD by $p < 0.001$ was demonstrated in subjects with KI values < 60% compared to the group with KI > 60%. The IOCD and KI, has a statistically significant effect on survival length of this patients group, so together they play a role as prognostic cofactors in the prediction of PR and survival length, on the basis of which an individual prognosis can be very effectively determined for each patient and may individualize the further pharmacotherapeutic approach.

DESIGN OF A NOVEL GlgE INHIBITOR VIA BIOISOSTERIC REPLACEMENT AND IN SILICO OPTIMIZATION OF AN EXPERIMENTALLY VALIDATED SCAFFOLD

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The GlgE maltosyltransferase is an important enzyme in α-glucan biosynthesis pathway in Mycobacterium tuberculosis. GlgE catalyzes the polymerization of maltose-1-phosphate (M1P) into linear α-glucan backbone. Inhibition of GlgE leads to toxic accumulation of M1P and bacterial death, making it a promising drug target. This study focuses on designing improved GlgE inhibitors using bioisosteric replacement and in silico screening. An experimentally confirmed GlgE inhibitor was used as the reference scaffold. Bioisosteric replacements were performed using the SwissBioisostere database, targeting either the phosphonic or glucopyranosyl moiety (one substitution per derivative). All newly generated ligands, along with the reference compound, were docked to the GlgE active site using AutoDock Vina on a supercomputing platform. Compounds with docking scores comparable to the reference underwent a second design round, combining top substitutions into hybrid molecules, which were redocked and re-evaluated. Physicochemical properties were assessed and compared to the reference ligand to identify candidates with improved drug-likeness and oral bioavailability. Among the tested substitutions, the glucopyranosyl moiety was successfully replaced only by a tetrazole fragment. In contrast, multiple fragments proved to be promising replacements for the phosphonic acid group, with the 3-methyl-1,2,4-triazole showing the most favorable results. Non-essential 4-hydroxyl group on the pyrrolidine ring was substituted with a fluorine atom which further improved predicted binding. This combination yielded a compound with binding affinity comparable to the original ligand. Novel GlgE inhibitor was found, with comparable binding affinity and significantly improved drug-likeness and physicochemical properties compared to the original scaffold.

PGX PROFILE AND PATIENT-REPORTED OUTCOMES OF ANTIDEPRESSANT DRUG THERAPY - DATA FROM THE ARTIPRO PROJECT

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Patient-reported outcomes (PROs) are increasingly crucial in evaluating drug therapies, reflecting patients' views on symptoms, adverse reactions (ADRs), and overall well being. This study investigates perceptions of antidepressant therapy efficacy and safety, and the relationship of pharmacogenetic (PGx) variants with PROs and ADRs. The study assessed 102 psychiatric patients using a questionnaire about current symptoms and possible antidepressant side effects. Of these, 28 patients had PGx profiles (ABCB1, ABCG2, COMT, CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP3A4/5, SERT) which were analysed with reported outcomes and side effects. Among 28 patients with full PGx data, sertraline (54.2%) and escitalopram (20.8%) were most frequently prescribed, with 92% receiving combination therapy, often with benzodiazepines. Common side effects were dry mouth (29.2%), increased appetite (25%), dizziness (20.8%), and sleeping disorders (33%). The distribution of CYP2C19 phenotypes: 50% normal metabolizers (NM), 25% intermediate metabolizers (IM), 16.7% rapid metabolizers (RM), 4.2% ultrarapid metabolizers (UM), and 4.2% poor metabolizers (PM). CYP2D6 phenotypes: 41.7% NM, 41.7% IM, 12.5% PM, and 42% UM. CYP2B6 phenotypes: 54.2% NM, 41.7% IM, and 4.2% RM. SERT (5 HTTLPR) phenotypes: 29.2% normal, 45.8% decreased, and 25% low function. CYP2B6 IM and CYP2C9 IM had a higher proportion of reported dry mouth and sleeping disorder. Patients with normal SERT function showed a better therapeutic response in motivation improvement. PGx profiles combined with POR provide complementary insights enabling more personalized and effective antidepressant therapy, reducing trial-and-error prescribing. Acknowledgements: The study is supported by the “Artificial intelligence for personalised medicine in depression – ArtiPro” ERA PerMed project.

CHOLINERGIC CONTROL OF MOTOR HYPERACTIVITY: INSIGHTS FROM A TETANUS NEUROTOXIN SPASTICITY MODEL

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Recent preclinical studies suggests that botulinum toxin type A (BoNT/A), a well-established antispastic agent, may exert effects at the level of central cholinergic synapses. In the spinal cord, propriospinal cholinergic transmission is mediated by C-boutons, which modulate motoneuron excitability via post-synaptic M2 muscarinic receptors. Potential role of endogenous cholinergic signalling in mediating sustained or intermittent muscle hyperactivity, particularly that resulting from disinhibition of ventral horn motoneurons, remains unclear. In the present study, we investigated the modulatory effect of spinal cholinergic transmission on experimental muscle spasms induced by tetanus neurotoxin (TeNT) a potent inhibitor of GABAergic and glycinergic synaptic transmission. To induce varying degrees of calf muscle spasm, rats received intramuscular injections of different doses of tetanus neurotoxin (TeNT). The involvement of spinal cholinergic pathways was investigated using low-dose intrathecal (i.t.) administrations at the lumbar level: the acetylcholinesterase (AChE) inhibitor neostigmine (5 μg/10 μL) and the M2 muscarinic receptor antagonist AQ-RA 741 (20 μg/10 μL). Muscle spasm intensity was evaluated by assessing resistance to passive dorsiflexion of the ankle, complemented by a series of motor and gait performance tests. TeNT administration into the gastrocnemius muscle produced a dose-dependent increase in muscle spasticity. Short-term blockage of spinal AChE augmented the severity of TeNT-evoked spasm, while blockage of spinal M2 muscarinic receptors resulted in its amelioration. Present results suggest that the spinal cholinergic transmission, associated with SNARE-dependent vesicular release of ACh and muscarinic M2 receptor signalling, augments the increased muscle tone evoked by lower motor neuron disinhibition. These findings reveal a new role for endogenous spinal ACh, with implications for the possible novel therapeutic approaches in sustained or intermittent muscle hyperactivity. Acknowledgement: The research was funded by Croatian Science Foundation (UIP-2019-04-8277; DOK-2021-02-6169).

DOSING OF RESERVE ANTIBIOTICS ACCORDING TO ESTIMATED GLOMERULAR FILTRATION IN PATIENTS WITH NORMAL AND REDUCED MUSCLE MASS

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A large number of reserve antibiotics are excreted by the kidneys, so it is very important to determine the correct dosage according to estimated glomerular filtration rate (eGFR) which is based on serum creatinine concentration. People with reduced muscle mass have lower serum creatinine concentration, so KDIGO guidelines recommend that in those people, eGFR is estimated by using cystatin C concentration. This study aims to determine the frequency of prescribing reserve antibiotics in people with reduced muscle mass and whether there is a need to introduce eGFR using cystatin C concentration with those patients. A retrospective study was conducted at the Special Hospital Varazdinske Toplice, which included patients who were prescribed at least one reserve antibiotic during 2024. Patient data were taken from the medical records and eGFR was estimated according to the CKD-EPI formula. The study included 115 patients who were prescribed 158 requests for reserve antibiotics. A total of 57 (49.6%) patients had reduced muscle mass and among them, only one patient had a reduced eGFR (< 60 ml/min/1.73m2). Of the 58 patients who did not have reduced muscle mass, 26 (44,8 %) had a reduced eGFR. The results of this research conclude that almost 50 % of patients who were prescribed reserve antibiotic had reduced muscle mass and that is necessary to introduce eGFR based on cystatin C for such patients in order to enable more adequate dosing of reserve antibiotics and reduce the risk of toxicity.

THE ASSOCIATION BETWEEN THE CHANGE IN CARBAPENEMS USE IN A UNIVERSITY CLINICAL CENTRE AND COVID-19 PANDEMIA

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Carbapenems, as broad-spectrum antibiotics, are reserved for severe infections. COVID-19 infection, particularly during the early stages of the pandemic, was linked to an irrationally high use of antibiotics. The aim of this study was to assess the change in carbapenems use during and after the pandemic.We have analysed the utilization of carbapenems in a tertiary care University Clinical Centre Nis, Serbia. The total yearly amounts of all three carbapenems used (ertapenem, meropenem and imipenem/cilastatin) were expressed as Defined Daily Doses (DDDs) per100 bed days. The 9-year period observed was divided into before (2016-2019.), during (2020-2022.) and after COVID-19 pandemic (2023-2024.). The median consumption of carbapenems before pandemic was 2.0 DDD/100 bed days, which significantly increased ($p<0.05$) during the pandemic (3.1 DDD/100 bed days) and after it (3.8 DDD/100 bed days). Meropenem, which accounted for 50.2% of carbapenem use before the pandemic, saw its utilization increase to 73.7% in the last two years, largely replacing ertapenem. The ratio of imipenem/cilastatin reached peak in the period 2020-2022 (33.3%), but after the pandemic, returned to the consumption before it. COVID-19 pandemic had a significant impact on carbapenems consumption, both in total DDD prescribed, and in the utilization of individual drugs. The increase in imipenem/cilastatin use during the pandemic and the continued increase in meropenem use, may have long-term harmful effects on resistance prevalence and the clinical efficacy of these antibiotics.

WHEN CURCUMIN MEETS ROSUVASTATIN: PRECLINICAL INSIGHTS INTO NOVEL SYNERGISTIC APPROACH TO HYPERLIPIDEMIA

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Given the potential to enhance pharmacological efficacy and reduce side effects, the management of hyperlipidemia has increasingly embraced a combinatorial approach that emphasizes the evaluation of synergistic pharmacological interactions between natural compounds and synthetic agents. The present study aimed to assess the efficacy of a novel synergistic combination of curcumin and rosuvastatin in a diet-induced hyperlipidemia model using Wistar rats, through the analysis of selected biochemical and hematological parameters. Sixty Wistar rats were assigned to five groups: (1) Standard diet, (2) Atherogenic diet, (3) Curcumin (atherogenic diet + curcumin, 200 mg/kg/day), (4) Rosuvastatin (atherogenic diet + rosuvastatin, 10 mg/kg/day), and (5) Combination (atherogenic diet + curcumin and rosuvastatin at stated doses). Hyperlipidemia was induced by administering the atherogenic diet for 14 days, followed by a 14-day treatment period. The serum lipid profiles, lipid ratios, hepatocellular injury markers, and markers of inflammation were evaluated. The following hematological parameters were determined: erythrocytes, leukocytes, platelets, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin concentration, and mean corpuscular hemoglobin. The combination of curcumin and rosuvastatin produced a more pronounced reduction in plasma lipid levels and lipid ratios compared to monotherapy, as well as in liver markers. Furthermore, the results indicate that experimentally induced hyperlipidemia exerts deleterious effects on hematological and inflammatory parameters, with significant alterations observed across multiple indices. Findings underscore the potential therapeutic benefits of combining curcumin and rosuvastatin in hyperlipidemia management; however, further preclinical investigations and well-designed clinical trials are warranted to validate these results.

NANOMATERIAL-MEDIATED MODULATION OF EXOSOMAL miRNAs IN ASTROCYTES: TOWARD NEW STRATEGIES FOR BRAIN TRAUMA TREATMENT

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Carbon nanotubes have emerged as promising nanomaterials for the treatment of neurological disorders due to their unique physicochemical properties and capacity to interact with neurons, neural circuits, and glial cells. When applied to culture medium, chemically functionalized single-walled carbon nanotubes (SWCNTs) modulate the morpho-functional properties of cultured astrocytes, cells that play a central role in the brain's response to injury, including after traumatic brain injury (TBI). The aim of this study was to evaluate the effects of SWCNTs on the expression of selected exosomal microRNAs (miRNAs) derived from primary mouse astrocytes. Primary mouse astrocytes were treated with chemically functionalized SWCNTs or vehicle. After 24 hours, culture media were collected for exosome isolation. Total RNA, including miRNAs, was extracted from isolated exosomes using the miRNeasy Tissue/Cells Advanced Mini Kit (Qiagen), in accordance with the manufacturer's instructions. Reverse transcription of mature miRNAs was performed using the TaqMan™ Advanced miRNA cDNA Synthesis Kit (Applied Biosystems). Quantitative real-time PCR was subsequently conducted using TaqMan™ Advanced miRNA Assays targeting miR-16, miR-21, miR-26, and miR-146. Preliminary results indicate that treatment with SWCNTs, applied as a colloidal suspension, induces changes in the expression of selected exosomal miRNAs released from primary astrocytes. These findings support further investigation of chemically functionalized SWCNTs as a novel nanomaterial-based strategy for modulating glial responses and intercellular signaling in the context of TBI.

PATIENT PERSPECTIVES ON THE THERAPEUTIC EFFECTS OF BOTULINUM TOXIN IN CERVICAL DYSTONIA

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Cervical dystonia (CD) is a chronic movement disorder characterized by involuntary contraction of cervical muscle of the neck. Botulinum neurotoxin type A (BoNT-A) is today considered standard of care for CD patients. The aim of our study was to investigate patient perspectives on the therapeutic effects of BoNT-A in CD using patient s on-line survey. The validated on-line questionnaire was distributed to dystonia patients through Dystonia Europe web site (from 2017-2019), Questionnaire was divided into three parts (I. General questions: name, age etc. II. Specific questions: type of dystonia, diagnosis etc. III. Therapy, quality of life, etc.). A total of 3120 patients completed on-line questionnaire. Of these, 1479 (48%) were CD patients (age 33-74 years), and majority were female (75%). Pain was the most common non-motor symptoms (75%) followed by depression (55%) and anxiety (50%). The most common treatment reported from 961 patients was BoNT-A (65%), followed by oral medication (25%) and physiotherapy (10%). Among patients treated with BoNT-A only 50% were very satisfied, 25% dissatisfied, 25% neutral. Sub-analysis showed that all satisfied patients reported significant reduction in pain. Only 40% reported significant improvement of work ability- But all wanted to continue therapy. This survey, involving the highest number of CD patients in EU, highlights that despite the regular BoNT-A treatment dystonia symptoms have significant impact on quality of life, daily activities and working status, BoNT therapy should be plan in advanced and discuss with neurologist to avoid high expectations and improve the satisfaction of patients.

COMPARISON OF BLEEDING RISK BETWEEN RIVAROXABAN AND APIXABAN IN THE TREATMENT OF ATRIAL FIBRILLATION

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Direct oral anticoagulants, including the factor Xa inhibitors rivaroxaban (RXN) and apixaban (APX) have improved clinical management of atrial fibrillation (AF) and venous thromboembolism. Though similarly effective, comparing safety and bleeding risk is important, especially in older, comorbid patients with polypharmacy. This study investigated bleeding events and their association with CYP3A4/5 and CYP2J2 variants in patients treated with RXN or APX. A prospective case-control study was conducted at the University Hospital Centre Zagreb, enrolling adult AF patients treated with RXN or APX for at least three months, with a follow-up period of twelve months. Patients experiencing bleeding events were classified as cases; others as controls. Genotyping for CYP3A4*22, CYP3A5*3, and CYP2J2*7 was performed using TaqMan real-time PCR. Clinical and laboratory parameters, comorbidities, and concomitant therapy were collected. Statistical analyses, including Hardy-Weinberg equilibrium assessment and chi-square testing, were performed using JASP 0.17.1. A total of 354 patients were evaluated: 177 on APX (median age=67 (20-89), m=109) and 177 on RXN (median age=69 (35-98), m=115). Bleeding events (serious=29, epistaxis=9, hematoma=3, hematuria=7, hemoptysis=2) were o in 22% of RXN-treated patients and 8% of APX-treated patients. Genotype distributions were consistent with Hardy Weinberg equilibrium. No significant association between CYP3A4*22, CYP3A5*3, and CYP2J2*7 variants and bleeding events was observed. This study confirms observations from real-world data that bleeding is much more common with RXN therapy than with APX. Further larger pharmacogenetic studies, exploring this and additional genetic markers, are necessary to elucidate individual bleeding risk and guide anticoagulant therapy optimization.

THE IMPACT OF ORAL CONTRACEPTIVES ON SALIVARY COMPOSITION IN WOMEN: A SYSTEMATIC REVIEW

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While oral contraceptives (OC) regulate reproductive hormones, their effects on saliva remain under-investigated, despite saliva's key role in oral health. This systematic review aims to investigate the effects of combined oral contraceptives on salivary female sex hormones, pH, flow rate, buffering capacity, and electrolyte concentrations in women. A systematic search of PubMed and Google Scholar was conducted to identify studies on salivary changes in OC users. Keywords included “oral contraceptives,” “saliva,” “hormones,” “pH,” and “electrolytes”. Human studies assessing at least one salivary parameter were analyzed. A total of 10 studies were selected, focusing on the potential changes in saliva composition influenced by hormones. The review revealed that oral contraceptive use is consistently associated with alterations in salivary composition. Most studies reported a reduction in salivary flow rate and pH, creating an environment favorable for acidogenic bacteria and increasing caries risk. Electrolyte shifts were also observed, including elevated calcium and sodium levels, while potassium and magnesium remained largely unchanged. Buffering capacity varied depending on the progestin type, with some users (e.g., levonorgestrel) showing higher bicarbonate levels. Additionally, the presence of estrogen and progesterone receptors in gingival tissues may contribute to increased inflammation and periodontal susceptibility in long-term OC users. Oral contraceptives may compromise the protective properties of saliva by altering its pH, flow rate, and mineral composition. These alterations may increase the risk of caries and periodontal disease in long-term OC users. Further longitudinal studies are necessary to confirm these associations and to develop targeted interventions.

CLOPIDOGREL CLEARANCE IN PATIENTS WITH ACUTE CORONARY SYNDROME – A POPULATION PHARMACOKINETIC ANALYSIS

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The pharmacokinetics of clopidogrel exhibits significant variability among patients, largely influenced by various pathological conditions. Despite the advantages offered by newer P2Y12 receptor blockers (including superior efficacy and reduced pharmacokinetic variability), clopidogrel remains important in the management of cardiovascular and cerebrovascular diseases. This study aimed to develop a population pharmacokinetic (PPK) model for clopidogrel clearance in patients with acute coronary syndrome (ACS). Using a non-linear mixed effects model (NONMEM), we analysed 25 parameters related to clopidogrel steady-state concentration and elimination in ACS patients, including various clinical and laboratory parameters, as well as concomitant medications. The target population consisted of 85 patients, aged 37-89 years (mean age 60.45±11.59 years). Most patients received a loading dose of either 600 mg (29.4%) or 300 mg (30.6%) of clopidogrel, followed by a maintenance dose of 75 mg once daily (90.6%). The evaluated mean population value for clopidogrel apparent oral clearance was 70.6 l/h, with an apparent volume of distribution of 432 l. The full model incorporated four factors: clopidogrel daily dose, total body weight, alanine transaminase (ALT) levels, and concomitant therapy with pantoprazole. The equation describing the mean population value of clopidogrel clearance in our cohort was: CL (l/h) = 130 + 5.1 x ALT. Our PPK model of clopidogrel clearance identified ALT as a significant covariate, underscoring the importance of heart damage in ACS on the absorption of clopidogrel.

COGNITIVE EFFECTS OF METHYLPHENIDATE IN A RAT MODEL OF SPORADIC ALZHEIMER'S DISEASE

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A link between Attention Deficit Hyperactivity Disorder (ADHD) in early life and later development of Alzheimer's disease (AD) has been established epidemiologically, with evidence from preclinical and clinical studies. We aimed to investigate cognitive effects of methylphenidate (MPH), a first-line treatment for ADHD, in the intracerebroventricular streptozotocin-induced (STZ-icv) rat model of sporadic AD using VLADISLAV, a custom home cage testing device. Adult male Wistar rats (n=40) were treated with STZ-icv (3 mg/kg) or icv citrate (CTR, control), split in two doses 48 hours apart, after which daily MPH treatment was initiated in a two-bottle regimen (4+10 mg/kg). 3 weeks after STZ-icv treatment, the VLADISLAV home cage operant system was used to assess the animals' learning rate, cognitive flexibility, and impulsivity. Over a 7-day testing period, the control (CTR) group achieved an average correct response rate of 85% by the third day, whereas animals in the STZ-icv group reached this criterion on the sixth day, indicating slower learning. The MPH-treated STZ-icv group reached the same criterion on the second day, similar to the CTR group, while CTR animals given MPH performed comparably to the untreated STZ group, both attaining the criterion on the sixth day. Notably, cognitive flexibility was reduced in the STZ group but remained unaffected in the STZ+MPH group. Furthermore, while impulsivity increased with learning in the other groups, it was reduced in the STZ+MPH group as the experiment progressed. The first-line ADHD drug methylphenidate ameliorates the STZ-induced cognitive deficit in the rat model of sporadic AD. As in humans, MPH shows detrimental effects in control animals.

NEUROPROTECTIVE EFFECTS OF DMT AGAINST 6-OHDA-INDUCED TOXICITY

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Parkinson's disease (PD) is the second most common neurodegenerative disorder, primarily characterized by motor symptoms. Although dopamine depletion is a key hallmark of PD, additional pathological mechanisms, particularly oxidative stress (OS), also contribute to disease development and progression. N,N-Dimethyltryptamine (DMT) is a plant-derived alkaloid known for its psychedelic properties. While it was previously regarded as neurotoxic, recent studies suggest that DMT may have neuroprotective effects, promoting neuritogenesis, spinogenesis, synaptogenesis, and anti-apoptotic pathways. We investigated the effects of DMT in SH-SY5Y neuroblastoma cells exposed to 6-hydroxydopamine (6-OHDA) for 24 hours. 6-OHDA is a commonly used neurotoxin in PD models, inducing cell death through mechanisms involving OS and apoptosis. We assessed the effects of DMT on cell viability (using the MTT assay and measuring ATP levels), oxidative stress (ROS levels), and cell death (lactate dehydrogenase activity, caspase-3/7 activity, and nuclear morphology via Hoechst 33342 and propidium iodide staining). Our results showed that high concentrations of DMT showed neuroprotective effects under conditions of severe cytotoxic effect of 6-OHDA, whereas under milder stress conditions, DMT exhibited cytotoxicity. The neuroprotective effect was supported by increased cell viability and ATP levels, along with reduced ROS levels, decreased lactate dehydrogenase and caspase-3/7 activities, and preservation of chromatin structure. These findings suggest that DMT may be a promising therapeutic candidate for PD. However, further research is needed to clarify its mechanisms of action and evaluate its safety.

HDF PROGRAM POPULARIZACIJE FARMAKOLOGIJE U MLADIH / HDF YOUTH PROGRAMME FOR PHARMACOLOGY POPULARIZATION

VASOPRESSOR-INOTROPIC SUPPORT IN PATIENTS WITH SEPSIS AND ACUTE KIDNEY INJURY: RETROSPECTIVE COHORT STUDY

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Sepsis-associated acute kidney injury (AKI) requiring renal replacement therapy (RRT) carries high mortality. Early initiation of vasopressor and positive inotropic support is critical for hemodynamic stabilization. This study evaluated patterns of vasoactive and positive inotropic drug use and their association with outcomes. The retrospective cohort included 49 ICU patients with sepsis and AKI requiring RRT. Exclusion criteria were death within 48 hours, ICU stay >28 days, end-stage organ disease, or incomplete records. Data were extracted from medical charts, including pharmacotherapy, hemodynamics, laboratory (arterial blood gas, lactate, bicarbonate) results, and clinical (7- or 28-day recovery/mortality rates and ICU/hospital stay duration) outcomes. Vasopressor-inotropic therapy was analyzed for drug type, timing, combinations, and duration. Systolic, diastolic, and mean arterial pressures at ICU admission were 107±34, 60±20, and 75±23 mmHg, respectively, while central venous pressure (n=39) was 13±5 mmHg. Vasopressor-inotropic therapy was administered in 96%, usually on day one. Noradrenaline was the main agent, often combined with vasopressin; multiple drug combinations were used in 20% of patients. Therapy duration averaged 9 days (IQR 5–14). Dobutamine was associated with lower 28-day mortality (OR 0.14, P=0.022), whereas adrenaline reduced recovery (OR 0.15, P=0.043) and increased lactate and central venous pressure. Vasopressin prolonged hospitalization (β=-14.06, P=0.038), and noradrenaline was linked to decreased systolic and mean arterial pressures. Early initiation of vasopressor-inotropic therapy is common in septic AKI patients on RRT. Dobutamine may improve survival, while adrenaline and vasopressin are associated with poorer outcomes or prolonged hospitalization, reflecting complex hemodynamic effects.

NEUROPROTECTIVE POTENTIAL OF A TAFAMIDIS-AMIDE PRODRUG: EFFECTS ON COGNITION AND BRAIN MOLECULAR CHANGES IN A MOUSE MODEL OF SPORADIC ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) necessitates new therapeutic approaches with disease-modifying abilities. Transthyretin (TTR) binds Aβ, prevents its aggregation, and promotes its clearance from the brain. Stabilization of TTR with small molecules such as tafamidis (TA) may enhance its protective role, offering a potential new direction in AD treatment, following amidation aimed towards enhancing its CNS-activity. Male C57BL/6J mice (3 months old, n=31) were randomly divided into 4 groups. sAD was induced via STZ-intracerebroventricular administration (6 mg/kg), followed by TA administration intraperitoneally (5 mg/kg) daily during 4 weeks. Following treatment, animals underwent behavioral assessment using standard tests (OF, MWM, EPM, T-maze, NOR), and brains were analyzed using immunohistochemistry and Western blot. Despite STZ's failure to induce the expected cognitive/behavioral impairments, suggesting strain-specific resistance and limiting functional evaluation of TA, molecular analyses revealed significant region-specific changes. In the HPC, TA reduced insulin resistance (evidenced by increased IR and IGFIR expression), normalized elevated p-ERK levels, alleviated neuroinflammation and oxidative stress (evidenced by reduced GFAP, SOD-1, and catalase expression), and mildly increased TTR levels, despite an unexpected rise in amyloid accumulation. Immunohistochemistry further confirmed that TA reduced Iba1-positive microglial activation, indicating a strong anti-inflammatory effect in this region. In the FC, TA increased NPY expression and reduced p-ERK and antioxidant enzymes, indicating a neuroprotective response. However, in the BS, TA exhibited a more complex and partially opposing effect. Tafamidis-amide demonstrated neuroprotective activity by attenuating neuroinflammation, enhancing insulin-related-signaling, and modulating stress kinase pathways, particularly in the hippocampus. Further studies using optimized disease models, dosing regimens, and longer treatment durations are warranted to characterize its therapeutic potential in AD.

THE EFFECT OF OLIVE LEAF EXTRACT (OLEA EUROPAEA) ON INFLAMMATORY RESPONSES OF BV-2 MICROGLIAL CELLS INDUCED BY LIPOPOLYSACCHARIDE

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Olive leaf extracts (OLEs) have demonstrated antioxidant effects and a reduction in inflammation in both in vitro and in vivo studies. The aim of this study was to investigate the effects of OLEs from three Croatian olive cultivars – Leccino, Buza, and Oblica – on BV-2 cells whose inflammatory response was stimulated by lipopolysaccharide (LPS). For the OLEs, an aqueous extraction (macerates) and an ethanolic extraction methods were used and the total phenolic contents were determined by Folin-Ciocalteu method and total flavonoids using the modified Dowd method. For the in vitro neuroinflammation model, LPS-stimulated BV-2 microglial cells were used. Cell viability and membrane damage were assessed through lactate dehydrogenase leakage assay. Microglial activation and polarization were assessed using the markers Iba1, iNOS, CD86, and CD206 by western blotting. Overall, ethanolic extraction method proved to be more efficient than aqueous extraction (total phenols ~3.2 to 3.7, total flavonoids ~2.8 to 2.7-fold higher contents). Total phenolic compositions of OLEs of three Croatian olive varieties showed that Leccino had the highest values in both aqueous and ethanolic extracts. Contrarily, it was Buza in macerates and Oblica in the ethanolic extracts that had the highest levels of total flavonoids. Additionally, LPS and the OLEs treatments caused various changes in the expressions of the microglial activation markers. In this preliminary study, OLEs modulated microglial activation, with ethanolic extracts of three Croatian olive cultivars showing higher phenolic and flavonoid content. Their potential as anti-inflammatory agents in neuroinflammation was also observed.

STUDENTS' PROJECT OF PRECLINICAL DRUG DEVELOPMENT: NONPEPTIDE PAC1 ANTAGONIST AS NEW POTENTIAL TREATMENT FOR MEDICATION OVERUSE HEADACHE

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Medication overuse headache (MOH) remains a significant public health challenge. This project aims to develop a novel, nonpeptide, oral antagonist of pituitary adenylate cyclase-activating polypeptide type I receptor (PAC1R) with the potential to alleviate MOH. After designing the molecule named flipropacipant, molecular docking experiments tested the affinity for PAC1R expressed on HEK293 cells. Aqueous solubility, CaCO-2 permeability, metabolic stability, and blood-brain barrier penetration were additionally tested. Pharmacokinetic profile was analyzed in female C57BL/6J mice following intravenous and oral administration. MOH was induced by continuous administration of rizatriptan to exacerbate nitroglycerine-induced cutaneous allodynia in female mice. Allodynia was tested using von Frey filaments, while anxiolytic-like behaviors were tested in Elevated Plus Maze, and Open Field Test. PAC1R localization, cAMP, and phospho-CREB levels were analyzed in the selected brain regions by immunofluorescence staining. Toxicological profile and addictive potential of flipropacipant, were evaluated according to the EMA guidelines. Flipropacipant demonstrated high affinity for PAC1R, slow dissociation rate, and good blood-brain barrier permeability. Toxicity studies revealed no observable side-effects or damage to any tissue in the two lowest and moderate tested doses. Pharmacokinetic profile was acceptable, with intensive hepatic metabolism (CYP1A2 oxidation and glucuronidation), and renal elimination. Behavioral results showed significant reduction of mechanical allodynia and slight reduction in anxiety behavior after acute treatment with the modest flipropacipant dose. Immunohistochemical analysis revealed less neuronal activation in the drug-treated group compared to control mice with MOH. Here we proposed development and evaluation of the pharmacological profile of the first-in-class PAC1R antagonist, named flipropacipant, as a promising therapeutic candidate for MOH. Favorable results would support further clinical development and first-in-human studies.

EFFECT OF HEATED TOBACCO PRODUCTS AND ELECTRONIC CIGARETTES ON SALIVARY FLOW RATE AND COMPOSITION IN COMPARISON TO CONVENTIONAL CIGARETTES

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Saliva plays a crucial role in maintaining oral health, and its quantity and quality can be significantly affected by smoking. This review compares the effects of conventional cigarettes, electronic cigarettes (e-cigarettes), and heated tobacco products (HTPs) on salivary flow rate (SFR), pH, viscosity, and immune components. A structured literature search was conducted until July 1st, 2025, using PubMed, MEDLINE, and Google Scholar. Inclusion criteria encompassed original human studies from the past 20 years focusing on the impact of smoking products on saliva, written in English, Croatian, or Serbian. A total of 20 studies were included (9 cross-sectional, 5 narrative reviews, 2 systematic reviews—including 1 meta-analysis—1 pilot study, 1 diploma thesis, 1 laboratory comparison, and 1 animal study). Conventional cigarettes were consistently associated with reduced SFR (7 studies), lower salivary pH (5 studies), increased saliva viscosity (3 studies), and decreased levels of immunoglobulins and antioxidants (4 studies). E-cigarettes also demonstrated negative effects: reduced SFR was observed in 2 studies and reduced pH in 2 studies, while one study confirmed elevated salivary cotinine levels. HTPs showed milder but similar alterations, with 2 studies reporting decreased SFR and 1 study reduced lysosomal and enzymatic components (lysozyme, lactoferrin). Overall, both e-cigarettes and HTPs negatively influenced salivary composition, though to a slightly lesser extent than conventional cigarettes. Although e-cigarettes and HTPs are promoted as less harmful alternatives, their impact on saliva suggests they still pose a risk to oral health. They exhibit effects comparable to traditional cigarettes, including reduced secretion, altered pH, and diminished immune defense. Due to limited long-term data, further studies are needed to fully assess their safety profile.

SMOKERS' VS. NON-SMOKERS' SALIVA: EFFECTS ON PLATELET-RICH PLASMA OPTICAL DENSITY

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Saliva contains endogenous substances that may influence platelet activity. Smoking can introduce exogenous substances into saliva that may affect platelet reactivity and may also have long-term effects on the oral environment. The aim of this study was to evaluate whether saliva from smokers and non-smokers affects the optical density of platelet-rich plasma (PRP) in a way that could be indicative of its activating effect on platelets. Unstimulated saliva was collected from 10 smokers (before and after smoking a cigarette) and from 14 non-smokers. In each well of a microtiter plate, 15 µl of saliva was mixed with 100 µl of PRP. Absorbance at 570 nm was recorded every 4 minutes for 20 minutes. Plates were shaken at 37°C for the entire monitoring period. PRP mixed with buffer served as control. Absorbance of pure saliva and salivary protein and calcium concentrations were also measured. No significant differences were observed between optical density of PRP mixed with buffer, non-smokers' saliva, or smokers' saliva over time. No difference was found between PRP mixed with smokers' saliva before and after smoking. Within-group analyses did not show an increase in light transmission over time. Correlation analyses revealed a positive association only between salivary protein concentration and the absorbance of pure saliva. Under the conditions of this study, no evidence was found that either endogenous salivary components or tobacco smoke constituents entering saliva exert an activating effect on platelets.

THE IMPACT OF TREATMENT BURDEN FROM INTRAVITREAL ANTI-VEGF INJECTIONS ON THERAPEUTIC OUTCOMES

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Intravitreal administration of agents targeted against vascular endothelial growth factor (anti-VEGF) represents a pharmacologically targeted approach to managing retinal vascular diseases such as neovascular age-related macular degeneration, diabetic macular edema, and retinal vein occlusion. Despite the high efficacy demonstrated in clinical trials, the real-world effectiveness is often reduced due to treatment burden, which influences pharmacotherapeutic adherence and persistence. This literature review was conducted using PubMed, Embase, Scopus and Web of Science, focusing on publications from 2010 to 2025. Search terms included: “intravitreal pharmacotherapy,” “anti-VEGF,” “treatment burden,” “adherence”, and “clinical outcomes.” Studies analyzing dosing frequency, patient compliance, pharmacokinetics of intravitreal agents and long-term outcomes were selected for analysis. Frequent intravitreal injections of anti-VEGF (often monthly) lead to substantial treatment burden, affecting adherence and resulting in therapeutic undertreatment. This discrepancy between pharmacological efficacy and clinical effectiveness is well documented. Patients receiving fewer than the recommended number of injections showed significantly worse visual acuity outcomes. Pharmacokinetic profiles of current agents (e.g., ranibizumab and aflibercept) necessitate frequent dosing due to limited intraocular durability. High treatment burden presents a significant pharmacological challenge, highlighting the need for agents with extended intraocular half-life and sustained drug delivery systems. Emerging therapies, such as port delivery systems and novel VEGF inhibitors with longer duration and/or dual mechanism of action (e.g., brolucizumab and faricimab), aim to optimize pharmacokinetics and reduce treatment frequency. Addressing these pharmacological limitations is essential to aligning real-world outcomes with clinical trial results.

KNOWLEDGE, ROLE AND RESPONSIBILITIES OF PHARMACISTS IN ASSISTING PROFESSIONAL ATHLETES TO PREVENT UNINTENTIONAL USE OF PROHIBITED SUBSTANCES

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Considering the sport as one of the highest evaluated elements of Croatian identity, the aim of this study was to explore the knowledge and skills of Croatian pharmacists in preventing the unintentional use of prohibited medications by athletes. Using a simulated-patient study design, the professional water polo player contacted by telephone 80 randomly selected public pharmacies from the four largest Croatian cities, excluding Split. Using a structured-interview protocol, advice on inhalation use of salbutamol (a WADA-prohibited substance with conditional requirements) for exercise-induced asthma was requested from the Masters of Pharmacy included in the study. Medical history-related questions asked by pharmacists, as well as advice given to the simulated patient, were recorded on the data collection form, and then coded and analyzed. Inappropriate advice regarding anti-doping was provided by 42.5% pharmacists, whilst two pharmacists (2.5%) completely withheld counsel, emphasizing that they did not know the answer or how to find it. The majority of pharmacists (70.0%) did not check any information about the patient's age, health status, or medications. Correct, comprehensive, and precisely communicated conditions for using inhaled salbutamol in sports were provided by only six pharmacists (7.5%). Community pharmacists in Croatia mostly lacked core anti-doping knowledge, as well as skills to properly screen sources of interest and translate the information into athlete counseling. This indicates the need for additional education in sport-related pharmacy in order to optimize care for this specific patient population, and to protect them from any type of anti-doping violation.

PHARMACOGENETIC INSIGHTS FOR OPTIMIZING DRUG THERAPY: IDENTIFYING AND MANAGING THERAPY PROBLEMS

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Adverse drug reactions (ADRs) are a common cause of morbidity and may result in therapy discontinuation or hospitalization. Pharmacogenetic testing identifies genetic variants influencing drug response, enabling individualized therapy and preventing drug therapy problems (DTPs). Integrated into Comprehensive Medication Management (CMM) in primary care, it supports early DTP detection and improved outcomes. This study examined referral reasons, documented DTPs, and evaluated how pharmacogenetic insights guided treatment. A retrospective interventional study was conducted at the Healthcare Centre Zagreb – Centar (Nov 2022–May 2025). Patients were referred for pharmacogenetic testing due to suspected ADRs or inadequate response. A clinical pharmacist reviewed history, medication use, and pharmacogenetic profiles to identify DTPs and recommend interventions using the Pharmacotherapy Workup method, guided by CPIC and DPWG. Follow-up included reassessment and evaluation of DTP resolution and clinical improvement. Eighty-three patients were referred, 56 (67.5%) tested; 69.6% were female, mean age 53.2 ± 17.7 years. At first consultation, 243 DTPs were identified (mean 2.93 ± 3.19 per patient), most commonly Needs additional drug therapy (42.8%), Dosage too low (11.9%), and Ineffective drug (2.8%). A total of 283 variants were detected (mean 5.1 per patient). Psychopharmaceuticals, mainly antidepressants (22.2%), accounted for most ineffectiveness, linked to CYP2C19, 5-HTTLPR, and CYP2D6. In 46.4% of patients, a variant was directly linked to a DTP; in 50.0%, findings led to interventions such as dose adjustment or substitution. Following intervention, DTPs were resolved in 44.6% of patients. Pharmacogenetic testing clarified DTPs and guided effective interventions, resolving nearly half of cases. Its integration into CMM could enhance medication safety and optimize outcomes.

INTRANASAL INSULIN RAPIDLY REACHES NASAL EPITHELIA WITHOUT ALTERING PERIPHERAL LEVELS IN FEMALE RATS

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The intranasal insulin (INS) route allows direct delivery to the central nervous system, minimizing side effects and enhancing cognitive function in patients with diabetes and Alzheimer's disease. However, the exact distribution of insulin within central and peripheral tissues is not yet fully understood. This study examines the time-dependent distribution and impact of INS in plasma, cerebrospinal fluid (CSF), as well as in the nasal respiratory (RE) and olfactory (OE) epithelia and the olfactory bulb (OFB). Female Wistar rats received intranasal insulin (2 IU) and were sacrificed at 3, 7.5, 15, 30, 60, and 120 minutes post-administration. Untreated animals served as controls. Insulin levels were measured in plasma, CSF, RE, OE, and OFB. Plasma and CSF insulin levels remained unchanged. In the RE, insulin spiked at 3 minutes and stayed elevated up to 15 minutes, after which it declined and remained at baseline for the other time points. In the OE, insulin peaked at 3 minutes before declining to normal levels by 30 minutes. In the OFB, no increase in insulin was detected. The findings demonstrate that intranasal insulin rapidly and efficiently distributes to the nasal epithelia, highlighting the nasal mucosa's potential as an effective absorption site even at minimal doses. The absence of significant changes in plasma and CSF insulin levels supports the concept that intranasal delivery primarily targets the CNS with minimal systemic exposure, reducing the risk of peripheral insulin-related side effects. Support: This work was supported by the Croatian Science Foundation under project number HRZZ-IP-2022-10-1895.

ANTIBIOTIC THERAPY IN PATIENTS WITH SEPSIS AND ACUTE KIDNEY INJURY: RETROSPECTIVE COHORT STUDY

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Sepsis-associated acute kidney injury (AKI) requiring renal replacement therapy (RRT) is associated with high mortality. Rapid initiation of antimicrobial therapy is essential. This study evaluated the frequency and patterns of antimicrobial drug use. A retrospective cohort of 49 intensive care unit (ICU) patients with sepsis and AKI requiring RRT was analyzed. Exclusion criteria were death <48 h, ICU stay >28 days, end-stage organ disease, or incomplete medical records. Data on pharmacotherapy and clinical (7- or 28-day recovery/mortality rates and ICU/hospital stay duration) outcomes were reviewed. A total of 30 different antibacterials were used, with patients receiving on average four agents (range 1–10). The most frequently prescribed drugs were meropenem (73%), vancomycin (59%), metronidazole (49%), linezolid (31%), and clindamycin (22%). Use of piperacillin–tazobactam was uniquely associated with higher recovery compared to other antibiotics (P=0.023). Antifungal therapy was given to 18 patients, while two patients received antivirals. Younger patients were more frequently treated with multiple antibiotics (r=–0.442, P=0.001). Patients receiving a higher number of antibacterials had significantly lower 7-day mortality (P=0.009; OR=0.457, 95% CI 0.216–0.963). This effect was not observed for 28-day mortality (P=0.649) or recovery rate (P=0.888). However, broader antibacterial therapy correlated with prolonged ICU/total hospitalization (r=0.494 and r=0.503, both P<0.001). In septic AKI patients undergoing RRT, early intensive antimicrobial treatment was widely applied, yet only piperacillin–tazobactam was associated with improved recovery. Overall, antibiotic therapy did not alter long-term mortality but was linked to reduced early mortality and longer hospitalization.

DISTRIBUTION OF BOTULINUM TOXIN TYPE A WITHIN THE TRIGEMINAL COMPLEX OF RAT FOLLOWING PERIPHERAL ADMINISTRATION

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Botulinum toxin type A (BoNT-A) is a potent neurotoxic product of the metabolic activity of Clostridium species, which blocks synaptic neurotransmission by inhibiting the formation of a functional SNARE complex. Although it was showed that BoNT-A exerts its antinociceptive effect at the level of the first synapse in the spinal cord, recent studies suggest the possibility of its trans-synaptic transport within the sensory system. Therefore, this study aimed to investigate the distribution of BoNT-A beyond the first synapse of the sensory system following its peripheral administration. BoNT-A (7 IU/kg) was unilaterally applied into the hind paw of male Wistar rats, one day before the intrathecal injection of either a specific antitoxin against BoNT-A or control horse serum. On day seven, carrageenan solution (2%) was bilaterally injected into the hind paws to induce inflammatory pain. Immunohistochemical analysis of cleaved SNAP-25 (cl-SNAP-25), a marker of BoNT-A proteolytic activity, was performed on sections of the principal trigeminal nucleus and the subnuclei of the spinal trigeminal nucleus. Signal intensity was assessed based on the presence and fluorescence intensity of cl-SNAP-25 immunoreactivity. Peripheral administration of BoNT-A resulted in the existence of cl-SNAP-25 signal on both the ipsilateral and contralateral sides across all examined nuclei. The strongest expression was observed in the principal trigeminal nucleus and the pars interpolaris subnucleus. Intrathecal administration of the specific neutralizing antitoxin, which binds only extrasynaptic toxin, significantly reduced the intensity of cl-SNAP-25 signal in all analyzed regions. The obtained results indicate retrograde axonal and trans-synaptic transport of BoNT-A from the peripheral site of administration to the supraspinal brain regions. These findings raise important questions regarding the further distribution of BoNT-A within the central nervous system and its entry into target neurons/cells. This research was funded by the Croatian Research Foundation (grants no. HRZZ UIP-2019-04-8277 and DOK-2021-02-6169, Croatian Science Foundation) and the project FarmInova (KK.01.1.1.02.0021) financed from the European Regional Development Fund, Operational Program Competitiveness and Cohesion.

INTRANASAL INSULIN ACUTELY MODULATES REDOX HOMEOSTASIS IN A REGION-SPECIFIC MANNER IN THE BRAIN AND NASAL EPITHELIA

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Understanding how intranasal insulin affects brain signaling and metabolism is essential for elucidating its therapeutic potential in neurodegenerative disorders with underlying metabolic dysfunction, such as Alzheimer's disease (AD). Oxidative stress (OS), which increases with aging, has been observed in both AD and type 2 diabetes mellitus, indicating a potential link between OS, brain insulin resistance, and cognitive impairment. This study aimed to investigate whether intranasal (IN) insulin acutely modulates oxidative stress markers in a region-specific manner. Male Wistar rats received IN administration of 2 IU of human regular insulin and were sacrificed 3, 7.5, 15, 30, 60, and 120 minutes after administration, six animals served as intact controls. Redox homeostasis (total antioxidant capacity (ABTS), lipid peroxidation (TBARS), low molecular weight thiols (LMWT), sulfhydryl groups (SH), and superoxide dismutase (SOD) activity was assessed in the hippocampus (HPC), hypothalamus (HPT), temporal cortex (TC), respiratory epithelium (RE), and olfactory epithelium (OE), and correlated with insulin signaling markers. Insulin decreased LMWT and antioxidant activity in HPC and HPT, while SOD activity increased in HPT, TC, and RE. OE and RE demonstrated increased antioxidant activity, with opposite LMWT and SH trends (decreased in OE, increased in RE). Lipid peroxidation was reduced in TC. No correlation between OS markers and insulin signaling was observed in HPC or HPT. SH correlated positively with IRS activity, while SOD was negatively associated with AMPK activity. In epithelia, LMWT and SH negatively correlated with C-peptide and positively with AMPK activity. Intranasal insulin administration induces region-specific alterations in oxidative stress markers, with distinct responses observed between brain regions and nasal epithelia. These findings highlight the complexity of insulin's redox effects and suggest a potential role of different brain areas and peripheral structures in mediating insulin-driven neuroprotection. This work was supported by the Croatian Science Foundation under the project number HRZZ-2022-10-1895 and University of Zagreb project (10106-24-1453). Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of Croatian Science Foundation.

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