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Future trends in health interventions

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ABSTRACT BOOK

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INAUGURAL LECTURE

IG. 1.

Re-conceptualizing the Modeling of Drug Addiction in Animals –

A Translational Perspective

Deroche-Gamonet V.

Université de Bordeaux, INSERM, Neurocentre Magendie, Bordeaux, France

Over the past 30 to 40 years, the clinical understanding of drug addiction, or severe Substance Use Disorder, has undergone significant changes. Consequently, animal models for studying drug addiction had to be re-conceptualized.

This lecture examines the transformation of animal models of drug addiction over the last 25 years, highlighting how this has enhanced our capacity to identify addiction-specific psychobiological changes and their translational value. It is now commonly agreed that the maintenance of drug seeking behavior despite negative consequences or the availability of natural rewards best models the maladaptive nature of addicted drug use. However, certain fundamental aspects of addiction, such as individual risk (where not all drug users develop addiction) and individual variations in the mechanisms that support addiction (where not all addicted users are alike), have been overlooked in animal models. The translational value of animal models of addiction could be further improved, and precision medicine for addiction could be better informed, by considering these two types of individual differences.

Animal models of higher brain dysfunctions have a crucial role to play in understanding the etiological processes of psychopathologies, which are difficult to access in humans. The questions that have arisen from animal models of addiction and the strategies that have been developed may also be applicable to other psychopathologies.

PLENARY LECTURES

PL. 1.

Molecular Mediators of Antidepressant Response

Turecki G.

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Major depressive disorder (MDD) is a prevalent condition with important associated morbidity and mortality. The first line treatment for MDD is provided with antidepressants. However, there is significant variability in antidepressant treatment response with over 40% of patients not responding to a single antidepressant trial and approximately 30% of patient not responding following multiple trials. Dr. Turecki's laboratory has been investigating molecular factors underlying antidepressant response using a combination of functional genomic approaches in both animal models and humans. During his talk, he will provide an overview of recent studies focusing on the effect of antidepressants on non-coding RNAs and their downstream modulation of the glutamatergic system. These studies identify new targets for intervention, and collectively, shed light into the mechanism that explain antidepressant response.

PLENARY LECTURES

PL. 2.

Defining the Path to Maximize Therapeutics for Substance Use Disorders

Cunningham K. A.

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North America is struggling with a continuing overdose crisis. Drug overdoses killed ~110,000 people in the U.S. (2022) with rising mortality associated with fentanyl contamination and psychostimulant abuse. This crisis is further complicated by the increasing incidence of substance use disorders (SUDs). Cocaine was involved in nearly 20% of overdose deaths in 2019; in 2020, ~1.4 million people reported current cocaine use disorder (CUD). While medications are available to facilitate recovery from alcohol, nicotine, and opioid use disorders, there are no approved CUD pharmacotherapies. Serotonin (5-HT) systems contribute to the pathobiology of CUD and several aspects of this system may be useful in increasing the precision of treatment of CUD. Dr. Cunningham will focus on serotonin target-phenotype relationships engaging neurocircuitry and molecular targets which precipitate CUD risk, particularly engaging 5-HT2AR neurobiology. Her talk will integrate published and unpublished data on novel 5-HT2AR agonists and potentiators and their distinct cellular and functionality in vitro and in vivo with the goal to maximize efficacy of targeted medications for these devastating disorders.

PLENARY LECTURES

PL. 3.

Endothelial Biomedicine and Experimental Pharmacology of Endothelial Dysfunction; A Call for Endothelium-Guided Diagnosis and Therapy

Chłopicki S.

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Vascular endothelium, covering the huge inner surface of the entire cardiovascular system, is recognized as a multifunctional organ that regulates a number of important functions to maintain cardiovascular homeostasis, while endothelial dysfunction contributes to the pathophysiology of multiple cardiovascular and non-cardiovascular diseases. All known risk factors impair vasoprotective mechanisms of endothelium, so endothelial function in *atherothrombosis* represents an integrated biological readout of individual response to atherogenic factors. Altered endothelial function is also present in cancer, locally in primary tumour, in metastatic-organs as well as in systemic vasculature contributing to the pathophysiology of cancer and cardiovascular complications of cancer treatment. Many other pathophysiologically distinct diseases are associated with altered endothelial function. Yet, there is no widely available, efficient methods to diagnose endothelial function and to monitor endothelial response to therapy in clinical setting of various diseases, whereby endothelial-guided therapy is needed.

In an effort to better understand the phenotype and mechanisms of endothelial/vascular dysfunction in various diseases, we developed the panel of methods for assessing endothelial phenotype *in vivo* (functional and biochemical endothelial profiling). Using such a unique *in vivo* approach, complemented with other methods, we were able to uncover some clinically relevant aspects of the pathophysiology of endothelial dysfunction in various murine models of cardiovascular diseases, endotoxic shock cancer metastasis or accelerated ageing as well as to profile endothelial response to cardiovascular or anti-cancer pharmacotherapy *in vivo*. Based on this experience accumulated over years, we increasingly appreciate the heterogeneous and complex nature of endothelial dysfunction that in some cases can only be experimentally recapitulated *in vivo*. It is a challenge to design mechanistically-oriented pharmacotherapy of endothelial dysfunction and apply in clinics, but endothelium-guided diagnosis and therapy is clearly called for.

PLENARY LECTURES

PL. 4.

Astroglial contribution to NMDA receptor activity

Oliet S.

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Understanding the properties of NMDARs and their regulation by the local environment is of paramount importance for the sake of our central nervous system. The possibility that the glycine-site of NMDARs could represent an important pathway to regulate NMDAR activity was disregarded for a long time based on the belief that this site was fully saturated by ambient glycine. We now know that glycine is not the only endogenous co-agonist of NMDARs and that both glycine and D-serine can differentially regulate the activity of these glutamatergic receptors. We will here review the latest results describing the key roles played by astroglial Ca^{2+}, IP_3 and membrane receptors in providing D-serine at excitatory synapses. The impact of these processes under physiological and pathological conditions will also be presented.

INVITED SESSIONS

Session 1: Blood-brain barrier dysfunctions in CNS disorders

S.1-1. Potential role of blood-brain barrier in stress resilience

Dziedzicka-Wasylewska M., Solich J.

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To study mechanisms of stress resilience we use three strains of mice differentially reacting to restraint stress (RS): mice with knock-out of the gene encoding norepinephrine transporter (NET-KO), which are well characterized as a stress-resistant phenotype as well as two other strains of mice displaying different stress-coping strategies, i.e., C57BL/6J (WT) and swiss SWR/J. We looked for markers of stress-resilience at the level of micro-RNAs (miRNAs) present in the serum, and we identified a group of miRNAs differentiating the stress response of NET-KO and SWR/J mice from WT animals. Among them were miR-1 and miR-155, as well as miR-324, miR-30, miR-19, miR-146, miR-200 and miR-214. Further analyses indicated that transcripts targeted by these miRNAs encode proteins responsible for BBB development and function. Using Custom TaqMan Gene Expression Array card we examined 29 transcripts in various regions of the mouse brain (prefrontal cortex, nucleus accumbens, hippocampus, and cerebellum). The most interesting results concern stress-induced down-regulation of mRNA encoding claudin5 in brain regions of all three genotypes. On the other hand, stress-induced alterations in the level of mRNA encoding occludin-1, caveolin, and TJP-2 are genotype- and brain region-dependent, what – given their role in maintaining BBB integrity – indicates BBB involvement in mechanisms leading to stress resilience. Some of these results were additionally confirmed at the protein level by immunohistochemistry.

BBB often gains more attention in the studies of brain diseases as an obstacle to be overcome in order to introduce various advanced medications inside the brain. But the integrity of BBB might be an important factor governing stress-coping strategies, which in turn are crucial in the context of depressive disorders, strongly associated with stress.

Acknowledgments: This study was supported by the National Science Centre Poland Grant No. 2016/23/B/NZ4/01086 and Statutory Activity of Maj Institute of Pharmacology Polish Academy of Sciences.

S.1-2. Sex-specific effects of early-life stress on blood-brain barrier function and response to immune challenge in rats

Chocyk A., Solarz-Andrzejewska A.

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Clinical data show that early-life stress (ELS) affects brain development and increases the risk of mental disorders and age-related cognitive impairments. Recently, blood-brain barrier (BBB) dysfunction and inflammatory processes have been highly implicated in the pathophysiology and progression of depression and neurodegenerative diseases. There are sex differences in the prevalence of the abovementioned disorders. The knowledge about the effects of ELS and sex on BBB function and inflammatory response is very limited. Therefore, we applied a maternal separation (MS) procedure from postnatal day 1 to 14 in rats to model ELS and studied its effects on BBB permeability and integrity, both in basal conditions and in response to lipopolysaccharide (LPS)-induced immune challenge in juvenile and adult males and females. To assess BBB permeability we injected a small-molecule tracer, sodium fluorescein, and determined its extravasated content in the brain. Additionally, we measured mRNA levels of tight junction (TJ) proteins and neuroinflammatory markers. We observed sex differences in BBB maturation and function. Adult females showed lower BBB permeability and higher mRNA expression of TJ proteins than males. MS increased BBB permeability in an age-, sex- and brain region-specific manner, both in basal conditions and in response to LPS. Interestingly, it was mainly accompanied by an increase in mRNA expression of TJ proteins. In basal conditions, MS specifically affected BBB permeability in juvenile males. Additionally, MS increased neuroinflammatory response to LPS in juveniles males. On the other hand, MS females showed blunted central response to LPS challenge mainly in adulthood. The results suggest that ELS may induce sex-specific adaptive or maladaptive changes in BBB functioning and inflammatory response, and in this way, precondition individuals to other environmental factors. These mechanisms may potentially underlie susceptibility or resilience to ELS-related disorders.

S.1-3. MRI Contrast Agents Crossing Blood-Brain Barrier

Błasiak B.

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The blood-brain barrier (BBB) is responsible for the movement of molecules, ions, and cells between the blood and the central nervous system (CNS), protecting the CNS from toxins, pathogens, inflammation and disease. While useful as protection, BBB disables effective treatment preventing most therapeutic drugs from entering brain tissue hence resulting in their lower efficiency. On the other hand, Magnetic Resonance Imaging (MRI), provides superior soft tissue contrast and is the best imaging diagnostic modality. One of the challenges in the MRI development is the production and application of contrast agents crossing BBB to deliver the contrast to the target, that often is protected by the barrier. This development led to the design of theranostic agents, that provide both MRI contrast and effective treatment of CNS disorders.

An example of such theranostic agents are agents based on nanoparticles (NP) that enable cellular tracking, targeted diagnostic, and image-monitored therapy. The advantages of using NPs as drug carriers include the improvement of stability and efficacy of hydrophobic drugs, biodistribution and pharmacokinetics characteristics, resulting in improved accumulation efficacy in the blood and targeted tissues. Development of new nano-delivery systems to the CNS, require surface modification of the NPs. An example of such modified NP, that targets the most aggressive brain tumors, gliomas will be presented. This is a primary brain tumor of glial origin, and 70% of glioma patients survive less than 15 months following symptoms, even with surgical excision and/or chemo-radiation therapy. To diagnose and treat this deadly disease, the DOX nanocarrier composed of Fe_3O_4 NPs and alginate, tagged with BBB-permeating G23 peptides on the particle surface was synthesized (G23-Dox/alg-Fe_3O_4 NPs) was developed. Tumors (U87MG) significantly shrank (from ~50 mm^3 to a few mm^3) in mice treated with G23-Dox/alg-Fe_3O_4 NPs after being intravenously injected with NPs for 5 days. This agent successfully crossed BBB, provided contrast in MRI and efficient treatment. Details regarding these types of theranostics will be provided.

Session 2: Mass spectrometry in medical sciences

S.2-1. The use of mass spectrometry in conformational diseases

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Background: Protein folding is crucial for proper cellular functions, whereas misfolding and/or protein aggregation are often associated with many neurodegenerative diseases. In this scenario, although many analytical and bioanalytical techniques have been recently applied by our group to monitor protein conformation and aggregation, mass spectrometry (MS) represents a unique and unreplaceable tool for investigating the biomolecular mechanisms involved in conformational diseases.

Materials and Methods: Various MS strategies have been applied in order to obtain information on enzymatic activities and/or conformational states of proteins involved in conformational diseases. Proteoforms of Tau (MAPT gene) harbouring ubiquitin (Ub) monomer installed at different positions (recapitulating proteoforms observed in post-mortem brain biopsies of diseased subjects) by di-sulphide coupling chemistry have been synthesized and tested for proteasome activity.

Results: Insulin-degrading enzyme as well as the 20S proteasome activity towards proteins with recognized pathogenic relevance in neurodegeneration and emerging roles in eye diseases have been unveiled and will be discussed.

Conclusions: MS is proved to be an important tool to study conformational diseases at molecular level.

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S.2-2. Next generation infection diagnostics

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Background: In response to WHO alerts, we recognize an urgent need to improve clinical diagnostics of the most significant pathogenic fungi, multidrug-resistant bacteria, and mycobacteria. We focus on the pulmonary, urogenital tract, and central nervous system infections.

Material and methods: In the next-generation infection diagnostics, we monitor microbial and mammalian metallophores at Microbe & Host interface. Our approach is based on Fourier-transformed ion cyclotron resonance and isotopically resolved mass spectrometry data filtering. On the molecular level, the concept monitors the partners quantitatively interacting in time during the tug-of-war for essential nutrients, i.e., during inflammation, invasive infection, or treatment and healing.

Results: As illustrated on filamentous fungi, siderophores (iron chelators) are produced in different phases of fungal pathogen development, from conidial germination to the stage of mycelial growth. They can be found in infected patients' distant or proximal fluids, e.g., urine or breath condensate, whereas they are absent or low in patients only colonized. The siderophore excretion in urine or breath condensate is compatible with noninvasive patient sampling at intensive care units and was used in monitoring fungal and bacterial coinfections. With pentraxin 3 (human acute phase protein) quantitation in bronchoalveolar lavage fluid, chronic pulmonary fungal and invasive bacterial infections were distinguished from invasive fungal diseases. If combined with fungal siderophores, mucormycosis can be distinguished from aspergillosis. Prompt silencing of siderophore production during an antimycotic therapy-moderated fungal infection in rat's lungs reflected the viability of a pathogen circulating in a host. We will provide biomarker cut-off definitions in equine infection diagnostics to support the one-health concept. On the bacterial note, rat brain tissue remodeling and E. coli dissemination in the brain were visualized with mass spectrometry imaging, scanning electron, and optical microscopies showing bacterial biofilm formation, with respective siderophores quantified in the brain tissue and cerebrospinal fluid.

Conclusions: The fair comparison of the performance of the noninvasive infection metallomics with current tools of invasive fungal infection diagnostics will be provided, and our future research pathways will be disclosed.

Acknowledgments: Czech Science Foundation (21-17044S).

S.2-3. Cholesterol and glucose analysis using the mass spectrometry imaging approach

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Background: Cholesterol is a very crucial molecule. About 25% of the whole-body cholesterol is synthesized de novo in our brain. Therefore, it means that the changes in the level of this molecule may be responsible for different pathological conditions. Glucose is the primary source of energy for neuronal cells, which indicates its crucial role in the brain's biochemistry. The brain is a very complex organ, and the possibility of retaining spatial information during its analysis is exceptionally important. MALDI MSI offers such an opportunity. Still, in the case of some molecules, analysis may be impossible if they do not ionize in the MALDI ion source.

Materials and methods: Cholesterol and glucose may be derivatized with betaine aldehyde. This compound reacts with the hydroxyl group of alcohols to form charged hemiacetal salt. The influence of the sputtering height of betaine aldehyde on the signal intensity and a number of solution layers were evaluated. Additionally, two different matrices for the final analysis were tested (CHCA and DHB). Tissue homogenates for quantitative analysis were prepared with different ways of homogenization. Appropriate amounts of cholesterol, glucose, and homogenate were mixed to prepare the spiked samples. Matrix made from the egg white was prepared to be able to create a calibration curve on the ITO slide for quantitative analysis.

Results: In our work, we have optimized the cholesterol derivatization by betaine aldehyde. Two parameters: the sputtering nozzle position over the sample and the number of betaine aldehyde solution layers, were optimized. Moreover, an exciting way of preparing a calibration curve with the aid of a white egg matrix was proposed.

Conclusions: In our study, we were able to perform quantitative cholesterol and glucose analysis in the rat brain cerebellum based on the betaine aldehyde derivatization and calibration curve obtained with the aid of spiked rat brain homogenate. We hope that our study will be helpful for those interested in cholesterol and glucose analysis.

Acknowledgments: This work was supported by the Polish National Science Center grant no. 2018/29/B/NZ4/02243

S.2-4. Possibilities and challenges of MALDI mass spectrometry imaging in cancer research

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MALDI mass spectrometry imaging (MALDI-MSI) is an analytical technique enabling qualitative and quantitative analysis of various chemical species in diverse materials with preserving information on their localization in an analyzed sample. Over the last decades MALDI-MSI has been gaining growing interest in the field of life sciences enabling imaging of endogenous biomolecules and xenobiotics in animal tissues. This has been accompanied with notable innovations in instrumentation, data analysis and sample preparation. In a typical MALDI-MSI measurement a tissue section is scanned with pulsed laser beam and the recorded mass spectra are correlated with histological structures within the tissue.

MALDI-MSI can be successfully applied to analysis of both fresh frozen and fixed tissues. Development of sample preparation protocols dedicated to formalin-fixed paraffin-embedded material has opened new era in MS-based molecular imaging of tissues especially in the field of cancer research, as it paved the way to analysis of well-documented samples of cancer tissues available in archives of pathology institutes.

MALDI mass spectrometry imaging has been employed in studies covering many significant issues not only in research on cancer biology but also in cancer diagnostics, including: tumor typing, grading and prognosis, tumor margin, tumor heterogeneity, hormone receptor status, prediction of response to treatment or identification of drugs and metabolites, among others. Along with constant progress in sample preparation procedures and instrumentation including data acquisition speed, achievable spatial resolution, and mass resolution, MALDI-MSI has emerged as a powerful, multiplexed tool to aid in cancer management.

However, one must be aware of some limitations of the technique which have to be addressed before MALDI-MSI becomes a part of routine cancer diagnostics. First of all, crucial aspects of method validation and standardization are still to be elaborated, although this problem has been recently gaining growing recognition. Furthermore, sample preparation and instrumental measurements still require a high level of expertise from researchers. Moreover, currently available high-throughput instrumental solutions generate large datasets requiring application of advanced computational methods for data analysis and large repositories for data storage. Hopefully, at least some of the above-mentioned problems can be expected to be solved within the next decade.

Session 3: Theranostic nanocarriers for drug delivery in central nervous system disorders

S.3-1. Progress of Engineered nanoparticles as theranostic nanocarriers

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Backgrounds: Theranostic nanocarriers have been considered as an emerging area for next generation of nanomedicine, capable of diagnosis, drug delivery and monitoring of therapeutic response. CeO_2 nanoparticles (CeNPs) have recently attracted increasing interests as regenerative and multi-enzymatic scavenging of reactive oxygen species due to the coexistence of oxidation states (Ce^{3+}) and (Ce^{4+}) and the reversible switching between these states.

In addition, Hybrid nanosilica (HNS) consists of organic inorganic silica nanoparticles with functional groups which can be used to attach compounds (Gd etc) for both therapeutic and diagnostic effects.

Materials and methods: CeNPs doped with Gd was used as theranostic nanocarriers bring both antioxidant capabilities and magnetic resonance imaging contrast enhancement. CeO_2 nanoparticles doped with Gd (CeO_2 /Gd) (MRI contrast) and carbon quantum dots (CeO_2 /CQDs) giving fluorescence were prepared through hydrothermal approach. Gd-HNS was prepared by a two-step synthesis: a controlled sol-gel synthesis of HNS NPs and followed by chemical conjugation with Gd.

The engineered nanoparticles will be characterized by XRD, TEM, UV-vis spectrometer and MRI to study their physiochemical properties including redox and MRI response.

Results: The crystalline size of the prepared nanoparticles is ca. 3 – 5 nm. Gd element was homogeneously distributed in the CeNPs. All the CeNPs (doped/undoped) exhibit antioxidating properties. Both CeO_2 /CQDs and CeO_2 /Gd showed photocatalytic activity. The lower T1 value for the CeO_2 /Gd water dispersion demonstrates the increased relaxation rate which was induced by the Gd element.

For the Gd-HNS particles, the MSME image presents contrasting properties of Gd-HNS NPs The areas with different signal intensity corresponding to different Gd concentration can be clearly distinguished. The NPs show similar performance as commercially used contrast agents.

Conclusions: CeO_2 doped nanoparticles with both antioxidant properties and increased magnetic relaxation rate are promising candidates as theranostic carries. Gd-HNS with comparable MRI response as commercial products can become potential theranostic nanocarriers due to their multifunctionality on their surface which can be further coupled with antioxidants.

Acknowledgement: The research leading to these results has received funding from the Norwegian Financial Mechanism 2014-2021, Thera4Nerv GRIEG Project 019/34/H/ST5/00578.

S.3-2. Understanding cellular structure: function relationships with super-resolution microscopy

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Super-resolution imaging has revolutionized the field of cellular biology by overcoming the diffraction limit of conventional light microscopy, enabling the visualization of cellular structures with unprecedented detail. However, most of the commonly employed super-resolution techniques require fixed samples, which limits insight into dynamic changes in both structure and function in living cells. To address this limitation, my group has recently employed genetically-encoded fluorophores on various calcium handling proteins. Here, live-cell imaging of protein positions are attained by photoactivated localization microscopy (PALM), with a resolution of ~40 nm. These images are then spatially paired with high-speed 2D imaging of calcium homeostasis. For example, a photoactivated red fluorescent protein (RFP) affixed to the Ryanodine Receptor type-2 has enabled live-cell analysis of this calcium release channel's positions in both cardiomyocytes and neurons. Rapid imaging of calcium has in turn revealed how neighbouring Ryanodine Receptors work together to generate unity calcium release events called calcium sparks. Similar analyses are in progress to examine positions and function of L-type calcium channels, and their crosstalk with Ryanodine Receptors. Examination of these relationships has significant implications for understanding human cellular physiology, through the use of induced pluripotent stem cells, but also how structure-function relationships are altered during disease. These approaches additionally have clear potential for tracking the position of fluorescently-labelled nanoparticles, to assess their real-time efficacy of drug delivery. Nevertheless, there remain clear challenges with these techniques. These include the at-present limited capacity to employ fluorescence imaging in the intact organ or organism, and to quantitatively "count" proteins since PALM imaging resolution remains below than the size of most proteins of interest. Addressing these challenges in future work will likely include emerging techniques for even greater imaging resolution, such as the MINFLUX technique (resolution ~2 nm).

S.3-3. Synthesis and characterization of polymer-based theranostic nanoparticles containing carnosic acid

Szczęch M.[1], Kruk T.[1], Łopuszyńska N.[1], Stachurski K.[2], Jasiński K.[2], Węglarz W.[2], Warszyński P.[1], **Szczepanowicz K.[1]***

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Background: Most new active substances are poorly water-soluble or even in-soluble, making them 'difficult to deliver.' Their poor solubility results in low bioavailability and low therapeutic concentration. Moreover, the toxicity of many drugs, their non-targeted delivery, and undesirable side effects result in low therapeutic effectiveness. The solution to these complicating problems is targeted drug delivery systems.

Materials and methods: The polymer-based theranostic nanocarriers were prepared *via* the nanoemulsion templating method. The nanoemulsion droplets were stabilized by docusate sodium salt/poly-L-lysine interfacial complex (AOT/PLL). Utilizing such nanodroplets as a template allows for preparing two types of nanocarriers, liquid core and polymeric core nanocarriers. The following polymers, i.e., poly(caprolactone) (PCL), poly(lactic acid) PLA, or poly(lactide-co-glycolide) (PLGA)) were utilized for the preparation of polymeric core. Prepared nanocores were further encapsulated in multilayered shells formed by the LbL adsorption of polyelectrolytes: poly-L-glutamic acid (PGA) and poly-L-lysine (PLL). The nanocarriers were modified for theranostic application.

Results: A procedure for the formation of polymer-based theranostic nanocarriers was proposed. Using AOT/PLL interfacial complex as a stabilizer of nanoemulsion droplets, we obtained nano-templates that allow the formation of two types of nanocores, liquid and polymeric ones. These nanocores were encapsulated by layer-by-layer adsorption of biocompatible and biodegradable polyelectrolytes. Gadolinum-labeled PLL and Rodamine-labeled PLL were used to form theranostic nanocarriers, while pegylated-PGA was used to form nanocarriers optimized for passive targeting. The average size of the obtained nanocarriers was 150 nm. The nanocarriers were stable for at least a period of 3 months. Model hydrophobic drugs were encapsulated in prepared nanocarriers.

Conclusions: Obtained nanocarriers loaded with model drugs are good candidates for further biological experiments.

Acknowledgments: This study was supported by the NCN project OPUS 20 2020/39/B/NZ7/01913 and by the statutory research fund of ICSC PAS.

S.3-4. Biosafety and neuroprotective potency of polymer-based theranostic nanoparticles containing carnosic acid

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Background: Nanoencapsulation of neuroprotective compounds and doping them with MRI contrast agents is a promising strategy for the new treatment of neurodegenerative diseases with concurrent monitoring of its targeting and efficiency. Our preliminary studies showed that among three chosen putative neuroprotective compounds (edaravone, ebselen and carnosic acid) screened in primary neuronal cell cultures against various harmful stimuli (oxidative stress, glutamate and oxygen-glucose deprivation), carnosic acid (CA) was the most effective against oxidative stress-induced cell damage. Therefore it was selected for encapsulation in polymer-based theranostic nanoparticles (NPs).

Materials and methods: Two types of polymer-based NPs (AOT/PLL-PEG and PCL-PEG) with and w/o gadolinium (Gd), empty and containing CA were tested for biosafety and neuroprotection assessment in primary neuronal cell cultures. For these purposes, two biochemical assays, MTT reduction and LDH release assays, were used. Cellular uptake of empty NPs labeled with rhodamine (Rd) in human neuroblastoma SH-SY5Y cells was estimated by flow cytometry.

Results: Empty and CA containing AOT/PLL-PEG NPs doped with Gd at dilutions 10- and 20-fold were more cytotoxic to primary neurons when compared to relevant NPs w/o Gd. We did not find any impact of the presence of CA in NPs on its biosafety profile which was similar to relevant empty NPs. The Gd-doped AOT/PLL-PEG and PCL-PEG NPs (empty and containing CA) showed in the MTT assay comparable cell damaging effects whereas in the LDH release assay, higher toxicity was found for PCL-PEG NPs. CA-containing AOT/PLL-PEG NPs, but not empty NPs, at dilutions 40- and 80-fold but not 160-fold, were protective against the hydrogen peroxide-induced cell damage. This effect was not affected by doping the NPs with Gd. However, CA alone at concentrations relevant to its content in NPs was not protective. We observed a similar time-dependency of cellular uptake of Rd-labelled AOT/PLL-PEG and PCL-PEG NPs in SH-SY5Y cells.

Conclusions: Both types of polymer-based theranostic NPs containing CA and doped with Gd showed comparable biosafety profiles and cellular uptake in neuronal cells. Moreover, an enhanced neuroprotective potency was evidenced for AOT/PLL-PEG NPs containing CA when compared to the effect of CA alone.

Acknowledgements: The study was funded by the Norwegian Financial Mechanism 2014-2021, Project number 2019/34/H/ST5/00578.

S.3-5. Polyacrylic acid (PAA) conjugated cerium oxide nanoparticles as efficient neuroprotectants against oxidative stress-induced cell damage in human neuronal-like cells

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Background: Increased oxidative stress is involved in the pathogenesis of various neurodegenerative diseases. Recently, nanoparticles with intrinsic antioxidant properties have gained attention to scavenge ROS in excessive inflammation and chronic injuries owing to their capability to switch between multiple oxidation states. Based on changes in redox state, cerium oxide nanoparticles (CeO NPs) have been widely studied for antioxidant activities, but their protective potential in Parkinson's disease has not been reported so far. Another issue is their safe and effective application as therapeutic agents due to agglomeration tendency. Hence, in the present study, we synthesised CeO NPs conjugated with polyacrylic acid (PAA) to improve dispersibility in an aqueous environment. The study aims at their neuroprotective effects in human neuroblastoma SH-SY5Y cells against the oxidative stress inducers, hydrogen peroxide (H_2O_2) and 6-hydroxydopamine (6-OHDA).

Materials and methods: The PAA-stabilized CeO NPs (PAA-CeO NPs) were synthesized by low-temperature precipitation followed by their characterization. Undifferentiated (UN) and 7-day retinoic acid (RA)-differentiated SH-SY5Y cells were used for cell culture assays. LDH release assay, propidium iodide staining/flow cytometry and light microscopy were used to measure cytotoxic effects, and caspase-3 activity assay was employed as an apoptotic marker.

Results: The size of synthesized PAA-CeO NPs was in the range of 50-60 nm. The XPS studies revealed details on the chemical composition and the level of oxidation of specific components. The developed particles at different dilutions were safe to both phenotypes of SH-SY5Y cells and 30 min pre-treatment of UN- and RA-SH-SY5Y cells with PAA-CeO NPs reduced the extent of cell damage evoked by H_2O_2 and 6-OHDA. Neuroprotection mediated by PAA-CeO NPs was not connected with direct inhibition of ROS production and attenuation of caspase-3 activity but was associated with attenuation of necrotic changes. Moreover, the nanoparticles tagged with FITC revealed a time and concentration-dependent cellular uptake.

Conclusions: The results point to the neuroprotective potential of PAA-CeO NPs against neuronal cell damage induced by oxidative stress inducers.

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Session 4: Modern approaches in immunodiagnostics and immunotherapy

S.4-1. Tale of two tubes exploring pathological mechanisms in systemic sclerosis - focus on monocytes and macrophages

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Systemic sclerosis (SSc) is an autoimmune disease characterized by aberrant activation of immune-cells. Monocytes actively contribute to pathogenesis of inflammatory diseases, including SSc. Pathway enrichment transcriptomics analysis indicated increased adhesion- and type-I-interferon (IFN-I)-dependent-genes in SSc monocytes. These cells displayed up-regulated expression of pro-adhesive CD11b/CD18, reduced anti-adhesive CD52 expression and enhanced adhesion to ICAM1 and endothelial cells. CD52 expression was consistent with SSc subtypes, immunosuppressive treatment and autoantibody profiles of SSc patients and monocyte adhesion properties. We demonstrated that down-regulation of anti-adhesive CD52 in $CD14^+$ monocytes represents a novel mechanism in the pathogenesis of SSc. Targeting of the IFN-HDAC-CD52-axis in monocytes might represent a new therapeutic option for early SSc patients.

SSc is also characterized by persistent overproduction of extracellular matrix and multiorgan fibrosis. The involvement of monocyte in SSc fibrogenesis remained poorly understood for decades. We showed accumulation of $CD14^+$ monocytes in collagen-rich areas and increased amount of alpha-smooth-muscle-$actin^+$ fibroblasts, $CD68^+/CD206^+$ macrophages in the myocardium, skin and lungs of SSc patients. Our transcriptomics findings identified activated profibrotic signature with elevated production of profibrotic fibronectin in $CD14^+$ monocytes and $CD14^+$ lung macrophages in SSc patients with interstitial lung disease (SSc-ILD) and highlighted the capability of $CD14^+$ monocytes to acquire a profibrotic phenotype.

Phagocytosis is a crucial cellular process, which under certain immuno-pathological conditions can be activated in macrophages in an uncontrolled manner. We recently demonstrated that human monocyte-derived macrophages (hMDM), differentiated from $CD14^+$ blood-derived monocytes from SSc patients, showed enhanced phagocytic activity compared to HC hMDM. Single-cell-RNA-sequencing analyses of lung macrophage populations identified upregulated phagocytosis-associated-*ARPC1B/ARPC2/ARPC5*-genes in $SPP1^{hi}/FCN1^{hi}/FABP4^{hi}$ macrophage clusters in SSc-ILD patients compared to HC. Similarly, single-cell-RNA-sequencing analyses of skin from diffuse-SSc showed increased expression of *ARPC1B/ARPC2/ARPC3/ARPC5/CD204/CD163/CD36* phagocytic-related genes in alternative-$CCR1^+$ macrophages compared to HC.Within a pool of genes enriched in lung and skin SSc macrophages, we observed a significant increase of upregulated pathways, including FcγR-mediated-phagocytosis, lysosome and regulation of actin cytoskeleton, and complement activation and defense responses to other organisms. Thus, targeting alternative/pro-phagocytic phenotype might be an effective tool to counteract SSc progression.

S.4-2. Future of cell-based therapeutics in the light of today's developments

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Despite the extensive progress of conventional medicine in the diseases' treatment, mostly due to the major advances in the field of pharmacology and diseases' early diagnosis and prevention, still large numbers of patients that have reached 'untreatable' stage are still awaiting for development of the effective treatment while they are a substantial burden to themselves, their families and the society.

Cell therapy is a particular medical filed where new, healthy and sometimes modified cells in a form of cell-based therapeutics are introduced into the body to replace diseased and damaged cells, to modulate the function of the patient's cells or to destroy the diseased cells.

Regenerative medicine combines together fields of medicine, science and economy, and is focused on developing new technologies that will enable disease treatment and improve patients' quality of live using more efficient and safe methods. One of the major themes of regenerative medicine is stem cell-based cell replacement therapy. Regenerative medicine is a very quickly developing, highly competitive field in modern medicine. Several approaches for using stem/progenitor cells in patient have been thus far considered; some have been tested pre-clinically and/or in clinical studies that usually involved rather small groups of patients and some have been already registered by authorization agencies.

Importantly, readily achievable (off-the-shelf) and highly effective cell therapeutics are yet to be determined. For instance, the embryonic cells or induced pluripotent stem cells are considerable usable sources of cells for regenerative medicine. Use of these cells, however, is associated with several ethical and technical problems. Another way to obtain stem cells for regenerative medicine is their isolation from adult tissues. Important drawbacks of adult stem cells use are their naturally low number, difficulties with harvesting and low expansion potential. In parallel to best cell's selection, optimization of clinically feasible delivery route(s) to achieve a maximized cell uptake in compromised tissue is a critical challenge that needs to be addressed to increase the efficacy of cell-based treatments.

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S.4-3. New directions in chimeric antigen receptor (CAR)-based immunotherapies

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Chimeric antigen receptor-T (CAR-T) cell-based therapy has become a successful option for treatment of several hematological malignancies, but also raises hope in a range of solid tumors and non-malignant diseases, as well. Indeed, CAR-based therapies are expected to revolutionize several areas of medicine, because of their potential to act as highly programmable "cellular scalpels", capable of removing any kind of "undesired" cells from the organism, if properly designed. In line with this statement, the future development of CAR-based therapies aims at coping with the most prominent challenges standing in front of this strategy, which are:

1. Choice of the suitable target molecule(s). 2. Heterogeneity of cancer cells. 3. Toxicity of CAR-T therapy, and 4. Immunosuppression of the tumor microenvironment. Overcoming these obstacles with the new approaches would allow CAR-based immunotherapies to transform the treatment market for a wide range of malignant and non-malignant diseases.

Session 5: The burden of neurodegenerative and psychiatric disorders: trends and future treatment

S.5-1. Turning over a New Leaf: Investigation of Endocannabinoid Regulating Enzymes in a Mouse Model of Chemotherapy-induced Pain

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Chemotherapy-induced peripheral neuropathy (CIPN) represents a debilitating side effect of cancer chemotherapeutic agents that often persists long after treatment. Here we tested whether inhibition of diacylglycerol lipase (DAGL)-β, an enzyme highly expressed on macrophages and microglia that produces the endogenous cannabinoid 2-arachidonoylglycerol (2-AG), would ameliorate nociception in a mouse model of paclitaxel (PAC)-induced nociception. The DAGL-β inhibitor KT109 fully reversed PAC-induced nociception in the von Frey assay of mechanical allodynia. Additionally, KT109 produced a conditioned place preference in PAC-treated animals, but not elicit a place preference or aversion in control mice. These findings suggest that DAGL-β inhibition reduces an aversive state resulting from PAC treatment, but lacks intrinsic rewarding effects on its own. KT109 also reduced the level of some cytokines and chemokines in dorsal root ganglia (DRG) taken from the L5-S1 segment of the spinal cord of PAC-injected mice. Because treatment of chronic pain conditions requires repeated dosing of analgesic or anti-inflammatory agents, we tested whether KT109 would maintain its antinociceptive effects or undergo tolerance following daily administration for six days. The antinociceptive effects of KT109 administered repeatedly were maintained at least 24 h after the last administration of the drug, whereas the antinociceptive effects of a single injection of KT109 persisted for less than 8 h. Moreover, repeated KT109 administration ameliorated PAC-induced hyperexcitability of primary afferent DRG neurons. Interestingly, PAC elicited mechanical allodynia in DAGL-α (-/-) mice and DAGL-β (-/-) mice, but DRG isolated from DAGL-β (-/-) mice showed protection against PAC-induced neuronal hyperexcitability. This pattern of findings suggests that DAGL-β blockade elicits complex and differential roles in ameliorating PAC-induced nociception. Finally, DAGL-β inhibition did not affect growth of A549 and H460 non-small cell lung cancer cells, and did not alter the antiproliferative nor the apoptotic effects of PAC in these cancer cell lines. Overall, these findings suggest that DAGL-β inhibition represents a promising strategy to treat neuropathic pain associated with chemotherapy.

S.5-2. Therapeutic use of psychedelics in mental health

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Background: A revamped interest for the study of hallucinogens has recently emerged especially for their potential application in psychiatric disorders. Considering how broadly psychedelics are being trialled clinically, surprisingly little is known about the mechanisms through which they act to elicit long term changes in behaviour and psychological state. Psychedelics exert complex biological actions, and a mechanistic understanding of these requires animal-based experimentation to examine psychedelic effects at a level of detail not possible in human studies and with the effects of expectancy entirely removed.

Methodology: By using behavioural pharmacology, electrophysiology, optogenetics, this talk will provide an overview of the neurobiological mechanisms underlying behavioural effects of psychedelics in animal models. The behavioural outcomes of interest span social and exploratory behaviours, anxiety-like phenotype as well as alcohol use disorder paradigms.

Results: Particularly, we will show that repeated administration with LSD (30 g/kg/day, for 7 days, i.p.) increases social interaction in mice ($p<0.001$), an effect that is paralleled to the modulation of the neuronal activity of the medial prefrontal cortex. Optogenetic photo-inactivation of the glutamatergic neurons in the medial prefrontal cortex reduces social interaction and inhibits the prosocial effect of LSD. In addition, we will show that the same regimen is able to prevent the development of an anxiety-like phenotype in stressed male rodents. Indeed, employing the chronic stress restraint paradigm, male mice display an anxiety-like phenotype in behavioural paradigm such as the open field test (OFT) ($p<0.001$), paralleled to a decreased neuronal activity of serotonergic (5-HT) neurons in the dorsal raphe nucleus (DRN)) ($p<0.005$). The repeated administration with LSD (30 µg/kg/day, for 7 days, i.p.) increases the time spent in the OFT and normalizes the decreased 5-HT DRN activity in stressed rodents. Finally, employing the drinking in the dark paradigm (DID), an approved model to induce alcohol use disorder, we will show that both single (150 µg/kg, i.p.) and repeated (30 µg/kg/day, for 7 days, i.p.) injections of LSD reduces the alcohol intake ($p<0.005$). Overall, this talk will give an update on the therapeutic findings in the field of psychedelic compounds in mental diseases.

S.5-3. Transcriptional effects of SSRI administration on brain networks

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The serotonin system is the main therapeutic target for antidepressant drugs, most notably the selective serotonin reuptake inhibitors (SSRIs). SSRIs such as fluoxetine are routinely prescribed as a first-line treatment for depression, as well as for a range of co-morbid conditions, however the drugs' mechanism is not well understood. In order to study the molecular and transcriptional effects of SSRI administration on their primary target serotonin neurons we performed spatial transcriptomics, a spatially resolved RNA-sequencing method in intact brain tissue. Brain sections containing the dorsal raphe nucleus and surrounding midbrain structures were sequenced using Visium spatial gene expression platform (10X Genomics), including from acute (single dose, 10mg/kg fluoxetine), chronic (22 days daily administration, 10mg/kg fluoxetine), and control treatment (single dose, saline) (n=3 per treatment condition, C57BL/6 mice). Both acute and chronic treatment induced a large number of changes in gene expression in the dorsal raphe nucleus. The Htr1a gene is upregulated after acute, and downregulated after chronic treatment, consistent with previous studies showing that blocking SERT results in downstream effects on 5HT1a autoreceptors. We also describe treatment-dependent transcriptional changes of neuropeptides thyrotropin-releasing hormone (TRH) and prodynorphin (PDYN) with specific spatial localization with the DRN, among others. Overall, our transcriptomic analysis reveals spatial and cell-type specific heterogeneity in SSRI action within the dorsal raphe nucleus.

Session 6: Sphingolipids in health and brain diseases

S.6-1. Sphingolipids in the brain: from memory control to psychiatric disorders

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Sphingolipids (SLs) are major components of cellular membranes in the brain. Thereby, ceramides and their precursor SLs, the sphingomyelins, are shaping membrane specialisations and lipid rafts that contain proteins involved in the specific signalling of designated neurotransmitter systems. The SLs of the brain are regulated by a plethora of enzymes, the SL-rheostat. Previous studies showed that the SL environment of a membrane is highly dynamic and, by that way, involved in normal behaviours and their plasticity in learning and memory. Here we discuss previous findings showing a role of acid sphingomyelinase (ASM) and ceramide in extinction learning of an operant response. Further evidence shows a brain area specific role of neutral sphingomyelinase (NSM) in learning and memory in a brain area specific way that gives rise to sex-differences in those behaviours. As learning and memory are the base for alcohol use and abuse behaviour, ASM as well as NSM play an important role in the control of emotional tone and subsequent alcohol consumption. This is mediated by multiple parallel acting pathways in the brain and in the bone-brain axis.

S.6-2. Sphingolipid-dependent membrane organization and signaling orchestrating myelin repair

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Recombinant human IgM22 (rHIgM22) binds to myelin and oligodendrocytes (OLs) and promotes remyelination in mouse models of multiple sclerosis. However, the target antigen and the signaling mechanisms through which rHIgM22 exerts its functions are still unclear.

We showed that 1) rIHgM22 binds *in vitro* to the glycosphingolipids (GSLs) sulfatide and lysosulfatide, but also to phosphatidylinositol, phosphatidylserine and phosphatidic acid; 2) changes in the composition of the lipid microenvironment of the target antigen can modulate the affinity of the antibody, suggesting that reorganization of lipid membrane microenvironment might be relevant in its biological activity.

In rat mixed glial cells (MGCs), rHIgM22 induced a dose-dependent proliferative, with the most significant response associated with astrocytes, and increased the production and release of sphingosine 1-phosphate (S1P) without affecting the total levels of ceramide. rHIgM22 treatment did not induce changes in the production and/or release of S1P in pure astrocyte or OPCs cultures, but it increased S1P release in BV-2 microglia cells, suggesting that rHIgM22 indirectly influences astrocytes proliferation via microglia-released S1P.

rHIgM22 had no effects on GSLs in MGCs and pure astrocytes, while in OPCs, OLs and BV-2 microglia we observe a significant increase in the levels of GM_3 and GD_3 gangliosides. In addition, we observed a significant decrease in cholesterol levels in differentiated OLs upon rHIgM22 treatment. No changes in phospholipid contents were observed in all cell types when treated with rHIgM22. Considering all this, we hypothesize that rHIgM22 myelin-repair activity could be at least in part mediated by alterations of lipid-dependent membrane organization in OPCs, OLs and microglia.

The finding that rHIgM22 treatment affected in opposite ways two sphingolipid metabolic enzymes further supports this hypothesis. rHIgM22 treatment in differentiated OLs reduced the activity of the acid sphingomyelinase (a key mediator for the detrimental effects of ceramide observed in mouse models of MS). On the other hand, S1P treatment in MGCs induced an increased expression of the galactocerebrosidase (known to be important to preserve the efficiency of myelin repair).

In conclusion, rHIgM22 exerts its protective effects by acting directly or indirectly on different glia populations involved in the mechanism of myelin repair, with sphingolipids being always key players.

S.6-3. Sphingolipid alterations in Huntington's disease: new insights into molecular mechanisms and potential therapies

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Huntington's disease (HD), a fatal genetic and rare neurodegenerative disorder, is characterized by a progressive striatal and cortical neurodegeneration, associated with motor, cognitive and behavioral disturbances. Among all the molecular mechanism defective in HD, perturbed metabolism of gangliosides, glycosphingolipids with a plethora of functions in the brain, has been reported to play a critical role in the pathogenesis of the disease.

In the last few years, our research group has extensively demonstrated that the metabolism of other sphingolipids, such as Sphingosine-1-phosphate (S1P), is defective in HD, even at an early stage of the disease. We have also shown that modulation of S1P axis is beneficial in different HD preclinical models.

More recently, we discovered an interesting link between the metabolism of S1P and the accumulation of glucosylceramide (GluCer) in HD pre-clinical models.

Our findings demonstrate that pharmacological interventions aimed at modulating S1P levels are able to normalize GluCer content in a HD animal model. This is associated with an amelioration of disease phenotype e with a modulation of neuroprotective pathways.

Collectively, our findings support the concept that the alteration of (glyco)sphingolipid pathways may contribute to HD pathogenesis and may be eventually pharmacologically targeted.

Our thought is supported by the evidence that some drugs, whose molecular targets belong to these pathways, are already in clinical trial for different other diseases and could be eventually repurposed for the treatment of neurodegenerative conditions and/or serve as a tool for the development of new ones.

Session 7: Innovative nanocarriers and treatment modalities in antitumor therapy

S.7-1. Reinvigorating antitumor immunity in brain tumors with short peptides and siRNA-nanocarriers targeting the tumor microenvironment

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Tumor cells stimulate molecular, cellular and physical changes within their host tissues creating a tumor microenvironment (TME), a complex and continuously evolving structure which supports tumor growth and progression. Its composition varies between tumor types, but hallmark features include accumulation of immune cells, stromal cells, blood vessels, and extracellular matrix. Antitumor immunity is inhibited or eluded by tumor-secreted factors that reprogram infiltrating myeloid cells and create the immunosuppressive TME. Advancements in single-cell techniques provide powerful means to systemically profile the multiple-omic status of the TME at a single-cell resolution, revealing the phenotypes and functionalities of disease-specific cell populations. Using single-cell RNA and protein sequencing (CITEseq) and spatial transcriptomics we identified the functional diversity and localization of immune cells in the TME of experimental malignant brain tumors. Microglia and peripheral monocytes massively infiltrate brain tumors and become polarized to promote glioma invasion, immunosuppression and angiogenesis. Computational analyses revealed interactions between tumor, myeloid cells and lymphocytes and pointed to some factors responsible for tumor-induced reprogramming of immune cells. The emerging pathways have been blocked with innovative peptides or siRNA and the effects of therapeutic interventions on TME reprograming and antitumor immunity have been assessed. Genetic manipulation of microglia in diseases using small interfering RNA (siRNA) was however hampered by the lack of safe and efficient siRNA delivery methods. We assessed the amphiphilic dendrimers (AD) for functional siRNA delivery and gene knockdown in primary microglia cultures. AD protected the siRNA from degradation and facilitated its cellular uptake. AD effectively delivered *Id1*-targeting siRNA to primary microglia and decreased target gene and protein expression. Id1 is a negative regulator of myeloid cell differentiation and is upregulated in glioma-stimulated microglia and *Id1* knockdown led to attenuation of microglia reprograming stimulated by glioma cells. AD complexes were also effective as *in vivo* siRNA-nanocarriers in orthotopic glioma model in mice. The results open up new perspectives in functional genomic studies and therapeutic targeting of microglia in brain tumors and other CNS diseases.

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S.7-2. Modular and adaptive dendrimer nanosystems in cancer treatment

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The application of nanotechnology is widely expected to bring breakthrough for cancer diagnosis and treatment. Dendrimers are ideal materials for elaborating nanomedicine by virtue of their well-defined structure, multivalent cooperativity and nanosize per se. I will present our recent studies on modular and adaptive dendrimer nanosystems, constructed via self-assembling of amphiphilic dendrimers, for the delivery of imaging agents, anticancer drugs and nucleic acid therapeutics in cancer detection and treatment. The self-assembling approach to create supramolecular dendrimer is completely novel in concept yet easy to implement in practice, offering a fresh perspective for exploiting the advantageous features of supramolecular dendrimers in biomedical applications.

S.7-3. Targeting of nanoparticles with homing peptides

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Our laboratory employs the in vivo phage display screening to identify homing peptides that selectively bind to specific targets in the vasculature. These peptides can then be synthesized and utilized to target drugs biologicals, and nanoparticles to tumors, thereby enhancing their therapeutic efficacy. I will outline our approaches to mapping vascular heterogeneity using in vivo peptide phage display, as well as our characterization and validation process for candidate vascular homing peptides. Additionally, I will provide examples of how we have used peptides and peptidomimetic compounds to target tumors and offer guidance on selecting homing peptides that are best suited for a specific disease model of interest. Lastly, I will present our unpublished data on blood-brain-barrier targeting peptides, which have the potential to deliver imaging and therapeutic payloads to glioblastoma and other neurological diseases. This innovative research could help to overcome the significant challenges associated with delivering therapeutic agents to the brain and has the potential to revolutionize the treatment of these devastating conditions.

Session 8: New targets, new ligands, new drugs: a novel approach for the treatment of the CNS pathologies

S.8-1. The role of microglial-derived extracellular vesicles in the pathogenesis of traumatic spinal cord injury: implication of exosomal microRNA

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One of the leading causes of long-term impairment is spinal cord injury (SCI). Primary injuries result in physical harm, while secondary injuries are characterized by neuroinflammation, neuronal death, and an overabundance of oxidants such reactive oxygen species (ROS). Due to their high amount of polyunsaturated fatty acids, rapid rate of oxidative metabolic activity, strong formation of reactive oxygen metabolites, and relatively poor antioxidant capacity, neurons are particularly susceptible to oxidative and electrophilic distress. In result, our in vivo bioluminescence results demonstrated Nrf2-ARE activation at the damage site and in a region of the brain during the first phase of SCI. Additionally, after SCI, we detected pathological alterations in the liver. As organs may interact with one another in part through the release of extracellular vesicles (EVs) and the absorption of them by micropinocytosis, organ cross-talk during SCI is still largely unexplained. As a method of genetic exchange between cells, EVs include a sizable amount of RNA that has been transported between cells. The pathophysiology of secondary SCI is significantly influenced by a group of endogenous, short, noncoding, single-stranded RNAs known as microRNAs (miRNAs). The number and composition of EVs undergo distinct changes as a result of cellular stress, which suggests that cargo loading into them cannot be a passive process. The regulation of the posttranscriptional processing and transport of exomiRs is mostly controlled by RNA-binding proteins (RBPs). SUMOylated heterogeneous nuclear ribonucleoprotein (hnRNP)-dependent processes were identified as one of the major potential pathways regulating RBP-RNA interactions and exomiR loading, according to recent studies. Although ADP-ribosylation, also known as PARylation, is involved in several cellular processes, its specific function in the interaction of hnRNPs with miRNAs is yet unknown. Our findings suggest that hnRNPA2B1 is a crucial RBP that selectively binds EVs miRNAs by identifying their unique patterns and regulates exomiR loading during SCI. Additionally, SUMOylation of hnRNPA2B1 in EVs regulates the protein's ability to bind to exomiRs.

S.8-2. Metabolic and reproductive dysfunctions associated with obesity and diabetes: From the womb to adulthood

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Background: Epidemiological and experimental data revealed the effects of a mother's nutrition on the development of offspring. The concept of foetal/early programming was proposed, according to which early environmental factors can permanently organize physiological systems in the offspring. An unbalanced maternal metabolic status may cause long-lasting physiological alterations, resulting in obesity and type 2 diabetes (T2DM). Besides metabolic problems in people and animal models of obesity and T2DM, there are numerous secondary problems, including reproduction dysfunction (alterations in sex hormones, miscarriages, infertility, etc.). An unbalanced diet rich in carbohydrates and fat and a lack of physical activity also contributes to obesity and T2DM in adulthood.

Material and methods: The following methods were used: induction of obesity by high-fat diet - HFD and CAF diet, and T2DM by HFD + streptozotocin injections, immunohistochemistry, RT-PCR, Western blots, pyrosequencing, assessment of metabolic and hormonal profiles.

Results: Sex-specific effects of the diets in prenatal and adult animal models of metabolic diseases on the hypothalamic-pituitary-gonadal axis, particularly concerning KNDy neurons localized in the hypothalamus and metabolic organs, were found. Moreover, sex-specific differences in immune and metabolic profiles were seen. The epigenetic mechanism was proposed, such as increased DNA methylation of Kiss1r promoter in the liver via which the diet affects metabolisms.

Conclusion: HFD and CAF diets contribute to both metabolic and reproductive abnormalities in animal models of obesity and T2DM.

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S.8-3. Emerging evidence for triclocarban as a neurodevelopmental risk factor: disruption of epigenetic status and dysregulation of neurogenesis-related genes

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Triclocarban (3,4,4'- trichlorocarbanilide) is a phenyl ether often added to personal and health care products, including cloths, plastics, and even products dedicated for newborns due to its antimicrobial activity. Although there is abundant evidence of triclocarban in human tissues, data available on the potential interplay of triclocarban with the developing mammalian nervous system are limited. It is known, that the exposure to environmental pollutants in utero during critical stages of development can have long lasting neurological outcomes. For that reason, in this study, we aimed to determine the effect of prenatal exposure to triclocarban on mammalian neurodevelopment with a particular emphasis on apoptosis, autophagy and epigenetic modifications. Also, to understand the impact of triclocarban exposure on neurodevelopment, the expression of neurogenesis- and neurotransmitters-related genes was measured.

Here, we used a model in which triclocarban was administered subcutaneously to pregnant mice at a dose of 5 mg/kg from the 15th to the 18th day of gestation. The following experiments were performed on brains of one-month-old mice (both sexes) which were prenatally exposed to triclocarban. We showed for the first time that triclocarban used at environmentally relevant doses targets the expression of apoptosis- and autophagy-related factors in sex-specific ways. The most interesting and disturbing fact is that prenatal exposure to triclocarban dysregulates the expression of a number of genes related to neurogenesis and neurotransmission and modifies the level of global DNA methylation in a sex-dependent manner. In males, triclocarban induces DNA hypermethylation and mainly affects neurogenesis pathways, while in females it induces DNA hypomethylation and affects neurotransmission pathways more strongly. Taking these data into account, we strongly suggest that triclocarban should be withdrawn from common use and to be categorized as a neurodevelopmental risk factor that may contribute to fetal neurological disease in adulthood.

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S.8-4. New treatment strategies against neural degenerations based on selective targeting of membrane estrogen receptors and PPARγ

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Alzheimer's disease (AD) and stroke have become the civilization diseases as their incidence has increased enormously in recent years. The diseases share a common etiology, including metabolic dysfunctions that increase the risk of their occurrence. Unfortunately, existing therapies are ineffective; rt-PA should be used no later than 4.5 h from the onset of the first symptoms of ischemic stroke, and anti-AD drugs provide moderate symptomatic relief but do not stop disease progression. Over recent years, PPARγ has been considered an attractive target for the treatment of the nervous system diseases, and membrane estrogen receptors (ERs) emerged as potential therapeutic targets to induce neuroprotection, avoiding detrimental side effects elicited by the activation of classical nuclear ERs. There is an urgent need to develop new therapies against AD and stroke. To address this issue, we assessed the neuroprotective capacity of the selective PPARγ modulator, amorfrutin B, and the pathway preferential estrogen, PaPE-1, which selectively activates membrane ERs i.e., mERα and mERβ. We were the first to show that amorfrutin B has a strong neuroprotective potential against hypoxia and ischemia that involves inhibiting apoptosis and autophagy as well as PPARγ hypermethylation and regulation of HAT and sirtuins activities. In addition, we demonstrated the neuroprotective effect of PaPE-1, which rescued mouse brain neurons from hypoxic and ischemic damage via inhibition of apoptosis and ROS formation. Importantly, amorfrutin B and PaPE-1 were effective when applied at 6-h post-treatment that position the compounds among the most promising anti-stroke therapeutics. Regarding AD, we showed for the first time that PaPE-1 elicited neuroprotection against amyloid β-induced apoptosis and oxidative stress. PaPE-1 restored mitochondrial membrane potential, attenuated caspase activities and downregulated *Fas*/FAS and *Bax*/BAX expression levels, thus preventing neuronal cell death. It is worth emphasizing that PaPE-1 was effective not only in co-treatment, but also in post-treatment paradigms that support its therapeutic efficacy in clinical settings.

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Session 9: Chaperons in Gaucher's disease

S.9-1. Current treatment options and novel strategies for lysosomal storage disorder, like Gaucher disease

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Gaucher disease is a rare inherited disorder caused by mutations in the *GBA1* gene, leading to a deficiency in glucocerebrosidase, and thus to an accumulation of glucosylceramide in macrophages.

There are three main types of Gaucher disease: type 1, the most common characterized by effects on the viscera such as splenomegaly and hepatomegaly, but also cytopenia and bone involvement; type 2, an acute neuronopathic form with severe neurological involvement; and type 3, a chronic neuronopathic form with variable neurological symptoms.

The diagnosis is confirmed by the demonstration of a deficiency of acid glucocerebrosidase in leukocytes.

Treatment options for Gaucher disease include enzyme replacement therapy (ERT), which involves regular infusions of the missing enzyme, or substrate reduction therapy (SRT), which reduces the production of glucocerebroside. These treatments are effective on visceral, hematological and bone manifestations but not on neurological symptoms.

S.9-2. Chaperone as a therapeutic modality in Gaucher disease

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Background: Biallelic mutations in the GBA1 gene, encoding lysosomal enzyme acid-beta-glucocerebrosidase (GCase), cause Gaucher disease (GD). This is due to decreased activity of GCase which leads to substrate accumulation, mainly in monocyte-derived cells. The disease is heterogeneous and its more severe forms involve neurological manifestations.

Mutant GCase, which is recognized as misfolded in the ER, is retained there for folding attempts. If not successful, the misfolded protein undergoes retro-translocation to the cytoplasm, ubiquitination and proteasomal degradation, in a process called ER Associated Degradation (ERAD). Retention of misfolded GCase in the ER initiates stress, which activates the stress response, know as the Unfolded protein Response (UPR). Chaperones are small molecules, with the ability to cross the blood brain barrier, that bind misfolded proteins in the ER to allow their correct folding and exit from the ER.

Methods: Using tissue culture cells, we have shown that the pharmacological chaperone ambroxol can decrease the ER retention level of mutant GCase and increase its lysosomal fraction and activity in a mutation dependent manner. We chose to use the fruit fly Drosophila melanogaster as an in-vivo model.

Results: We documented that expression of mutant human GCase in the fly activates UPR. If expressed in dopaminergic cells, it leads to their age-dependent death, motor deterioration and decreased survival. All these parameters can be alleviated by the addition of ambroxol, or by the chemical chaperone, arimoclomol. Arimoclomol promotes natural folding of nascent proteins and refolding of misfolded proteins by increasing the folding capacity of the ER.

Conclusions: Our results strongly argue that the fruit fly is a convenient model to test the efficacy of different chaperones in removing misfolded GCase from the ER and ameliorating UPR associated constaints.

S.9-3. Chaperone treatment option in neuronopathic GD and GBA related Parkinson

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Gaucher disease (GD), one of the two most common lysosomal storage disorders (LSDs), is caused by bi-allelic mutations in the glucocerebrosidase gene (GBA1) leading to reduced activity of the glucocerebrosidase enzyme, and hence to the accumulation of the substrate glucosylceramide (GC) in macrophages throughout the body, mainly in the spleen, liver and bones. Accordingly, the two currently available therapeutic modalities are enzyme replacement therapy (ERT) and substrate reduction therapy (SRT). Despite the success of both types of medications, not all of the disease manifestations are effectively abrogated by ERT and SRT and none of them cross the blood-brain-barrier to impact on the neuronopathic forms of the disease.

The realization that in addition to glycolipid storage GD is also a protein misfolding disorder, has opened the door to a third therapeutic modality – pharmacological chaperones. The protein misfolding leads to endoplasmic reticulum (ER) stress, to the unfolding protein response (UPR) and to early ER associated degradation (ERAD) –all of these processes contribute to the different neuropathological changes related both to neuronopathic GD and to its relation to Parkinson disease (PD). It also explains why GBA1-related PD develops not just in patients with GD but also in carriers.

The pharmacological chaperones are capable of partially removing the misfolded proteins from the ER, thereby relieving ER stress, avoiding ERAD and preventing consequent complications. Ambroxol hydrochloride, an over-the-counter antitussive drug available in many markets, was found in 2013 by Mahurn and Maegawa to act as a pharmacological chaperone for mutant glucocerebrosidase, albeit in higher doses compared to those used for cough.

My presentation will summarize safety and efficacy data in all three types of GD, focusing on the reversibility of some of the key neurological symptoms in types 2 and 3, which have inspired its use in GBA1-related PD and its potential not only to become a disease modifying agent in newly diagnosed patients, but also to actually prevent GBA1-related PD among individuals at risk, those GD carriers who present or develop significant PD prodromal features.

Session 10: Tracking brain projections and cell signaling involved in neuroadaptations triggered by cocaine, stress, or neurodegeneration

S.10-1. Investigating the link between habitual cocaine seeking and punishment resistance in rat models of addiction

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Background: Addiction is characterized by continued drug use despite negative consequences. In an animal model, a subset of rats continues to self-administer cocaine despite footshock consequences, showing punishment resistance. We sought to test the hypothesis that punishment resistance arises from failure to exert goal-directed control over habitual drug seeking.

Material and methods: Male and female rats were trained on a seeking-taking chained schedule of cocaine self-administration and then given 4 days of punishment testing, in which footshock was delivered randomly on one-third of trials. Several days before and after punishment testing, we assessed whether responding was goal-directed or habitual using outcome devaluation via satiety.

Results: We found that habitual cocaine seeking was not predictive of punishment resistance. However, punishment resistance was associated with persistence of habitual responding, whereas punishment sensitivity was associated with increased goal-directed responding after punishment (even if animals showed habitual responding prior to punishment).

Conclusions: These findings indicate that punishment-resistant cocaine seeking is related to a dominant habit system that persists under conditions that should encourage a transition to goal-directed control over behavior.

Acknowledgements: Funded by the National Institute on Drug Abuse.

S.10-2. Multimodal differences between projections of the ventral pallidum and relevance to cocaine reward

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Background: The ventral pallidum (VP) is a central hub in the brain reward circuitry involved in drug seeking behavior. The majority of the neurons in the VP are GABAergic but recent evidence suggest that different VP projections have different behavioral roles. This may be attributed to their different downstream connectivity but these neurons may differ in other aspects as well. Here we aimed to examine whether VP projections to 4 downstream targets – the mediodorsal thalamus (MDT), the lateral habenula (LHb), the lateral hypothalamus (LH) and the ventral tegmental area (VTA) – originate in different neuronal populations with different input sources, gene expression, excitability and relevance to cocaine reward.

Materials and methods: We injected WT C57 mice with a retrograde virus expressing either a fluorescent marker or Cre recombinase in each of the 4 targets of the VP. To examine the monosynaptic inputs to each VP projection we used the rabies method; to analyze gene expression in each projection we used RiboTag mice, which allow pulling out mRNA from Cre-expressing neurons; to test the excitability we used whole-cell patch clamp recordings from retrogradely-labeled VP neurons; and to examine the effect on cocaine reward we used inhibitory DREADDs to inhibit each projection while testing the preference for a cocaine-paired side.

Results: Our data show that the 4 VP projections show differences in all aspects described above. Primarily, the VP projections to the LH and to the VTA seem to originate from different cell populations – they show only 7% overlap; they differ in the proportions of their striatal, amygdalar and olfactory inputs; they differ in their excitability (the projections to the LH being much more excitable than those to the VTA); they express different sets of genes, including genes relevant to excitability; and they affect cocaine preference differently – inhibiting VP projections to the LH reduces cocaine preference while inhibiting VP projections to the VTA enhances it.

Conclusions: Our data show that different VP projections also differ in other aspects and may represent largely separate populations of neurons. Understanding the differential properties projection neurons, in the VP and generally in the brain, may be crucial to understanding the role of different projections in various behaviors.

Acknowledgements: Supported by the U.S.-Israel Binational Science Foundation.

S.10-3. Psychosocial crowding stress affects neuroplasticity-related signalling pathways within rats' frontal cortex in a region-dependent manner

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Background: Difficulty in adapting to stress leads to hypofrontality and the development of stress-related disorders including anxiety and depression. Recently we found that the procedure of psychosocial crowding stress (CS) does not enable the habituation of animals to stress conditions and affects glutamate signal transduction in the frontal cortex (FC) similarly to models of depression. Our study aimed to perform the behavioral characteristics of rats exposed chronically to CS. Moreover, we analyzed the mRNA expression of β(1-3)AR, GluA1 and their downstream effectors in FC to assess the impact of CS on signalling events engaged in neuron remodeling. Additionally, we checked CS effect on the level of GABA signaling molecules: parvalbumin (PV), GAD67, GAT1.

Material and methods: Male Wistar rats were overcrowded (70 cm2 per rat) for 3, 7, or 14 days while the control were kept under standard conditions (312 cm2 per rat). Open Field, Elevated Plus Maze, Novel Object Recognition, and Social Interactions tests served for behavioral study. Molecular analyses utilized RT-qPCR and immunohistochemistry methods and were performed in two adjacent FC loci: the medial prefrontal cortex (mPFC) and primary motor cortex (M1).

Results: CS rats exhibited anxiety depressive-like behavior and visual memory deficits. Profile of changes in mRNA level of studied molecules depended on the time of stress exposure and FC region. In M1, the expression of β3AR and GluA1 mRNAs were increased in the CS7d group, while PV and GAT1 were decreased in CS7d and CS14d groups vs. control. CS14d did not affect the number of PV cells. In mPFC, the mRNA level of β3AR, GluA1, kinase FAK, phosphatase STEP, GTPase Rac1, and RapGEF3 was decreased in the CS14d group vs. control. The number of PV cells in mPFC was slightly decreased in the CS7d group; however, the mRNA level of GABA markers was unchanged.

Conclusions: Our results revealed differences in the CS effects on the intracellular signaling in two adjacent FC regions. Restricted to mPFC decreased level of intracellular signaling molecules, engaged in neuronal remodeling, can be involved in the mechanism of stress-evoked hypofrontality. Affected by CS the expression of selected GABA signalling molecules point out PV interneurons in M1 as a significant locus to study the mechanism of hypofrontality.

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S.10-4. Utilization of conditional single lentivirus CRISPR/Cas9 vector for efficient gene knockout generation in adult neurons in vivo and in vitro as potential new model for study prodromal phase of Parkinson's disease

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Background: It is now well known that the neurodegenerative process in Parkinson's disease (PD) is divided into stages, with damage to various locations occurring before neuronal loss in the substantia nigra (SN), being directly a pathophysiological hallmark of PD. Clinical data show that degeneration of noradrenergic neurons of locus coeruleus (LC) is associated with PD and may precede the loss of dopaminergic cells. Here we developed Cre-controlled, lentiviral CRISPR/Cas9-based transgenic tool for targeting Cre-expressing noradrenergic neurons in DBHCre mice. The aim of this study was to trigger progressive degeneration of LC neurons and exploit this new model in the context of early, pre-symptomatic phase of PD.

Material and methods: In vitro efficiency of our unique vector LVV-CRISPR-DiO was tested on primary neurons cultures. During stereotaxic surgery of 9-weeks old DBHCre mice, LVV-hSyn-CRISPR-DiO vector was administered to the LC to silence the expression of Rrn3 (gene encoding transcription factor TIF-IA, controlling polymerase I activity) to selectively trigger degeneration of noradrenergic neurons. Mice were characterized by behavioral tests and spontaneous neuronal activity in SN.

Results: Silencing Rrn3 resulted of neurons death after 7 days in vitro. In vivo, we achieved a progressive degeneration of 20 % of LC neurons along with behavioral phenotype. HPLC analysis performed in striatum, a brain structure with strong projection of noradrenergic and dopamine neurons, showed no changes in dopamine metabolites, but revealed lowered level of noradrenaline in male mutant mice. Also, decreased gene expression of NET (noradrenaline transporter) in hippocampus proved the efficiency of mutagenesis by 61%. Electrophysiological studies have shown desynchronization of spontaneous activity of dopamine neurons. Also, proteomic analysis in SN revealed mitochondrial impairment which is an important determinant of processes at the forefront of PD.

Conclusions: Cre-controlled CRISPR/Cas9 mutagenesis revealed to be an effective approach in targeting TIF-IA protein as a tool to trigger progressive neurodegeneration associated with human neurodegenerative diseases. Changes in the functioning of the noradrenergic system are reflected in related structures, such as the SN, which can directly affect the susceptibility of dopamine cells to neurodegeneration in PD.

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Session 11: Medical biotechnology in disease modelling, drug research and therapy

S.11-1. Stem cells in neurological diseases modelling and therapies: possibilities, hopes and hypes

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Properly chosen stem cells capable of differentiation into relevant neural cells can be used for disease modelling and drug research, but also for repair of the nervous system. Regarding the latter, in common view, cellular therapies have enormous promise for regeneration of damaged tissues and organs. However, neither the human adult brain cortex nor the spinal cord contain stem cells and do not regenerate. Therefore, foetal neural stem cells or properly differentiated pluripotent stem cells, i.e., embryonic stem cells or induced pluripotent stem cells (iPSC) are investigated for therapeutic potential in Parkinson's, Alzheimer's, Huntington's diseases, spinal cord injury or amyotrophic lateral sclerosis. On the other hand in some leukodystrophies, gene therapy-corrected hematopoietic stem cells (HSC) can partially restore the production of the missing enzymes. In addition, in multiple sclerosis, the HSC, if properly administered, could potentially be considered due to the autoimmune character of the disease. In general, clinical trials of cellular therapies demonstrated some benefits of approaches in neurological diseases. However, they face technical and medical challenges, as well raise the ethical concerns when unproven cellular interventions are offered prematurely or without sufficient medical rationale.

In this talk, the generation of patient-specific iPSCs for modelling of Duchenne muscular dystrophy, spinal muscular atrophy, and amyotrophic lateral sclerosis will be described. Examples of biologically justified approaches to treating some neurological diseases will be presented. Furthermore, the discussion will also be devoted to unjustified cellular interventions, based on dubious cells, such as Wharton's jelly fibroblasts of the umbilical cord, offered without scientific rationale, and frequently commercially, to numerous patients suffering from very different conditions. This global problem will be discussed from the perspective of Poland where it reached an alarming level. These activities, in addition to affecting patients who are unaware of their scientific and medical status, are also dangerous to the development of rational stem cell therapies.

Conflict of interest: none

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S.11-2. From NMJ on a single chip to a Simplified Miniature Arrays (SMA) for drugs screening

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Spinal Muscular Atrophy (SMA) is characterized by the loss of the alpha motor neurons (MN), leading to neuromuscular junction (NMJ) impairment and muscle atrophy. Our goal is to model this pathology using human derived-induced pluripotent stem cells (iPSC) in a new personalized high-throughput drug-screening device to test their ability to rescue SMA-NMJ dysfunction. Samples from patients were used to generate pathologic iPSCs as well as their isogenic controls counterparts, (engineered by CRISPR-Cas9 to restore the defective gene). MN and myoblasts derived from these iPSCs have been first co-cultured in one individual microfluidic device to recreate in vitro human NMJ. The final platform will contain 32 of these chips coupled with custom Micro Electrode Array (MEA), to record electrical activity. First, we characterized the IPSC-derived cells and assessed the functionality of resulting NMJ by calcium imaging and electrical activity. Then, we have created prototypes to miniaturized the chip and cultured the NMJ in the 32-chips-platform to ensure system reproducibility. We are currently testing, different designs of MEA to adapt the electrodes to the devices and select the most relevant for overall electrical activity recording. This model should provide a platform for drugs screening and discover new therapies able to restore electrical activity of the pathological NMJ.

S. 11-3. The therapeutic effects of induced pluripotent stem cells and their exosomes on cardiovascular diseases

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Background: Induced pluripotent stem cells (iPSCs) have great therapeutic potentials for degenerative diseases including cardiovascular diseases. However, stem cell therapy is yet to be widely translated into clinical practice, mainly due to its several significant limitations. Each of the limitations, including differentiating into unwanted cells, short migratable distance and immunogenicity, may block most of the therapeutic potentials.

Materials, Methods and Results: Using 53 experimental conditions and diseased models, we first demonstrated that intravenously or topically disseminated application of iPSCs in vivo is feasible and can be safe. We then adopted multiple strategies to receive purer and more vital endothelial cells, myocardial cells and their progenitor cells, respectively. iPSCs, endothelial progenitor cells, iPSC-derived exosomes and engineered artificial heart tissues inhibited arterial neointimal proliferation, promoted angiogenesis and decreased infarct area in non-human primate, pig and rodent models of atherosclerosis, transplant vasculopathy, naturally aged vessels, would healing and myocardial infarction, respectively. Appropriate genetic modification may improve survival, vitality, differentiation, safety and therapeutic function of iPSCs and their derivatives. For example, we found integrin β1 (Intgβ1) was the dominant integrin β unit in iPSCs that mediates the adhesion of circulatory iPSCs and endothelial cells (ECs) as well as adhesion between iPSCs and extracellular matrix. When administered intravenously, Intgβ1 knockout or Intgβ1-siRNAs inhibit iPSC adhesion to and migration across activated endothelial monolayers. iPSCs, iPSCs transfected with nontargeting siRNAs, and endothelial-prone stem cells selectively homed on the luminal surface of aterial allografts, differentiated into ECs, and decreased neointimal proliferation while cells with Intgβ1 knockout or Intgβ1-siRNA transfection did not. In contrast, when administer topically, Itgb1-knockout increased iPSC's migration, angiogenesis- and the wound-healing-promoting effects.

Conclusions: Multiple strategies for safer, more efficient cell therapies using iPSCs and their derivatives for cardiovascular diseases were successfully developed.

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Session 12: Novel rapid-acting antidepressants

S. 12-1. Emerging antidepressants for the new age

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Background: A new age of antidepressant medications has emerged with the introduction of rapid-acting antidepressants with efficacy in treatment-resistant patients.

Materials and methods: The newly approved medicines and those in clinical development for major depressive disorder (MDD) and treatment resistant depression (TRD) are reviewed from the clinical literature, clinical trial registration data, and company website documents.

Results: New medicines based upon the model of ketamine have been delivered and are in clinical development. In just the past few years, novel antidepressants have been introduced for patient use that include (S)-ketamine, brexanolone, and dextromethorphan/bupropion. Newly emerging antidepressants are currently in clinical development that include (R)-ketamine, (S)-methadone, and the novel NMDA receptor antagonists Gate-202 and Gate-251. Other novel glutamate receptor modulators targeting mGlu and AMPA receptors are also in development. The later include TP0473292 (mGlu2/3 receptor antagonist prodrug) and TAK-653 (AMPA receptor potentiator). Psychedelic compounds including those without psychedlic effects are in clinical development by a plethora of biotech companies. Additional GABAA receptor modulators are also in direct line for clinical development such as Zuranolone.

Conclusions: The new age and antidepressants raises hope for clinicians and patients. However, there is still much to learn about these medicines and about best use practices. Rapid onset and the ability to impact TRD by these drugs raises the question of best first-line medicines for patients – Can we by-pass the weeks- or months-long progression currently used with standard of care drugs? Although the arresting of disease progression is likely a more futuristic goals, new age antidepressant researchers are already reaching for improved patient care – faster acting, more robust response and remission, and take-home therapies with gusto combined with a low burden of adverse events given dosing as prescribed. Predictive biomarkers for response to these drugs, research ongoing now, could enable an MDD patient to carry a small diagnostic kit and a s.c. injector pen in their pocket as diabetic patients do now. How is that for a Brave New World?

Acknowledgements: We are grateful to the Henry and Nellie Pence Foundation for their continued support of efforts to identify and develop improved medications for those who continue to suffer.

S. 12-2. Molecular signature of ketamine response/non-response in a preclinical model of depression

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Background: Stress is a key risk factor in the onset of neuropsychiatric disorders, including depression. Accordingly, the Chronic Mild Stress (CMS) rodent model of depression is widely used in the preclinical setting to study both etiopathogenetic and antidepressant mechanisms. The individual response to stressful stimuli may induce adaptive or maladaptive changes, respectively leading to stress resilience or vulnerability. Ketamine has recently emerged as the first rapid-acting antidepressant drug effective in patients with treatment-resistant depression. However, the molecular mechanisms underlying ketamine response/non-response are still largely unknown.

Material and methods: CMS was applied for 5 weeks on male rats, sucrose preference test was used to evaluate the anhedonic phenotype, and acute subanesthetic ketamine (10 mg/kg) was intraperitoneally injected to stress vulnerable animals 24 h before sacrifice. The hippocampus was collected to obtain DNA, RNA and proteins. Transcriptional changes were evaluated by RNA-seq analysis. DNA methylation profile will be evaluated by Reduced Representation Bisulfite Sequencing (RRBS), and total as well as synaptoproteomic analyses will be performed by mass spectrometry.

Results: The sucrose preference test allowed to classify the rats as stress resilient or vulnerable and ketamine responder or non-responder. Transcriptomic analysis revealed specific changes in the different experimental groups. Enrichment analysis showed differences between control and vulnerable rats and between ketamine responders and non-responders in the expression of genes involved in pathways such as glutamatergic synapse, synaptic signaling and organization, modulation of neuronal and dendritic spine morphology.

Conclusions: The integrated results of this study should help us to better elucidate ketamine rapid mechanism of action and resistance.

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S .12-3. Ketamine-like antidepressant potential of mGlu2/3 receptor antagonists and progress of other candidates targeting the glutamatergic system

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Background: Recently, glutamatergic systems have attracted much attention as targets for the development of novel antidepressants, which mimic the antidepressant profiles of ketamine. Among glutamatergic systems, I have focused group II metabotropic glutamate (mGlu) receptors, consisting of mGlu2 and mGlu3 receptors, because of their modulatory roles in glutamatergic transmission and distribution in the brain.

Materials and methods: Similarities in the antidepressant profiles and mechanisms between ketamine and mGlu2/3 receptor antagonists are presented, based on the preclinical findings obtained to date. Moreover, progress of other candidates targeting the glutamatergic systems is presented.

Results: Accumulating evidence has indicated that mGlu2/3 receptor antagonists have antidepressant-like effects in rodent models that mirror those of ketamine. Thus, mGlu2/3 receptor antagonists have been shown to exert rapid-acting and long-lasting antidepressant-like effects in rodent models, and to be effective in rodent models which are refractory to conventional antidepressants. In addition, mGlu2/3 receptor antagonists also share underlying mechanisms at synaptic and network levels with ketamine that are presumably responsible for these antidepressant-like actions. Based on these findings, Taisho is developing TS-161, a prodrug of TP0178894 (an mGlu2/3 receptor antagonist). TS-161 was demonstrated to be orally bioavailable and extensively converted into TP0178894 in a phase I study. Moreover, after oral administration of TS-161, enough amount of TP0178894 was found in the cerebrospinal fluid to block mGlu2/3 receptors and exert the anticipated antidepressant effects. A phase 2 study of TS-161 is currently underway in patients with treatment-resistant depression. In addition to mGlu2/3 receptor antagonists, clinical studies of several compounds targeting the glutamatergic systems (NMDA receptor antagonists/modulators and an AMPA receptor potentiator), a stereoisomer of ketamine (arketamine) and its metabolite (2R,6R-HNK), are ongoing, and some compounds have been proven efficacy.

Conclusions: It is expected that novel antidepressants that are safer, but as potent and rapidly acting as ketamine would be generated from drug discovery and development activity of post-ketamine agents.

Session 13: Digital medicine - beyond diagnostics

S. 13-1. Medical 3D cardiac anatomy supported by Mixed Reality

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Background: With increasing quality and resolution in medical imaging the 3D reconstruction of organs becomes within clinical reach. Medical imaging provides many 2D (DICOM) views on the 3D body, leaving the 3D interpretation to the medical experts. Recent developments enable the 3D reconstruction of organs with many available segmentation tools. Although segmentation software provides such capabilities, for clinical practice and education such tools are too complex to be used. To train medical students and staff to deal with these advanced medical imaging based reconstructions, an easy to use tool and educational material needs to be developed.

These 3D reconstructions provide many advantages in clinical evaluation, diagnosis and preprocedural planning. However, there are no standard clinical tools to provide a 3D segmentation alongside with the medical imaging. Such tool could bring the 3D segmentation a step closer to the clinical workflow and thus improve the clinical diagnostic, prognostic and procedural planning within a clinical workflow. The development of an easy to use imaging/3D segmentation model tool opens a new way to teach this complex anatomy of the heart and the procedures executed on the heart. In this project educational cases using the aimed educational tool will be developed.

Methodology: Multimodality approach to 3D case analysis can be divided into stages:

- 3D visualization of source data, 3D Medical Imaging Techniques
- 3D visualization of data presented as a 3D model - semi-automatic segmentation (from raw data to 3D model)
- Preparation of source data - and building a database of clinical cases - complementary source data - acquisition of medical data that allows them to be used to prepare 3D model
- Building a Clinical Case Reports Database to Facilitate Case-Based Learning and Improve Critical Thinking of Medical Students and Medical Staff
- Clinical cases showing the 3D anatomy of the heart, taking into account the multimodality used in a given diagnostic process (coronary angiography, CT, MRI, ECG, etc.), as an educational material useful in the process of educating interdisciplinary Clinical Staff

Results: Use the built platform for clinical purposes preoperative planning as an example of everyday practice use is presented.

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S. 13-2. Novel Ways of Bringing Non-Invasive ECG Imaging into Clinical Practice

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Background: ECG imaging is a non-invasive technique using multimodal data for reconstruction of cardiac electrical activity. A typical workflow for such a procedure includes the following steps: recording of body surface potential maps (BSPMs) with a multi-channel system, CT or MRI acquisition followed by segmentation of heart and torso together with electrodes positions, solution of the inverse problem for a source representation of interest.

Material: To date, numerous scientific, both technical and clinical, studies revealed potential benefits of bringing such a technology to the electrophysiological (EP) lab and led to formation of several companies offering commercial devices. However, achieved methodological advances do not yet fully meet the clinical needs in multiple aspects.

Conclusions: In our analysis, the associated hindering factors will be discussed in detail and some alternatives for overcoming the status quo will be presented. These include, in particular, integration of AI-based methods for automatic segmentation of cardiac chambers, advanced ECG classification, solution of the inverse problem, and possibility of real-time application.

S. 13-3. The Ventricular CineECG during Acute Ischemia

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Background: CineECG provides 3 different views on the standard 12 lead ECG: a) the PathECG which relates the cardiac activation and recovery trajectory through the hear, b) the delta maps, showing the comparison results to a normal database, and c) the differences of the ECG waveforms and PathECG over time. In the study the CineECG visualizations are created from the ECGs recorded during acute myocardial ischemia and changes in ventricular activation and repolarization during acute ischemia were quantified.

Methods: The PathECG provided the location and direction of the ventricular activation and recovery over time, using a reference 3D heart/torso model. The recorded ECG during percutaneous transluminal coronary angioplasty with prolonged balloon inflation from the first thirty patients in the STAFF III database were used as study sample. ECGs at baseline and at every subsequent 10 seconds during the first 150 seconds of balloon inflation were analyzed with CineECG. For the terminal QRS-complex, ST-segment and

T-wave, the direction of the CineECG was determined. The changes in direction relative to the direction at baseline were quantified by calculating the *Δangle* between the CineECG direction at baseline and the direction after every 10 seconds of inflation. The root mean square amplitude (rmsA) of all ECG leads at the ST-segment (10-60 ms) was computed for every selected median ECG beat.

Results: The median *Δangle* for the terminal QRS-complex was highest after 150 seconds after the start inflation (55.9° [26.2 - 88.9]). The median angle of the ST-segment and T-wave were highest at 29.6° [8.0 - 42.9] and 34.3° [13.2 - 60.1] within the first 10 seconds after start inflation, respectively. The median rmsA of the ST-segment was still small (0.05 mV [0.03-0.07]) at this point, which was a 16% increase from baseline. The median rmsA was highest after 180 seconds of inflation (0.09 mV [0.07-0.17]), which was a 136% increase from baseline.

Conclusions: The novel CineECG views prove to be very sensitive in detecting early changes in the ECG caused by the balloon inflation, even before significant changes occur in ST-amplitude. This implies that CineECG is able to pick up subtle ischemia related changes in the ECG.

S. 13-4. Future trends in human brain atlasing

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Background: Making maps and atlases is an ancient art-science. Through millennia mankind has produced maps of surrounding matter, initially land and sea, later planets and solar systems, and recently also the human brain. Brain atlasing has progressed from hand-drawn cortical maps to print atlases and recently, thanks to remarkable developments in acquisition techniques and computing, to advanced brain atlas platforms.

Material and methods: This work is based on a literature review and my three-decade-long contribution to the field. I defined the human brain atlas as "a vehicle to gather, present, use, share, and discover knowledge about the human brain with a highly organized content, tools enabling a wide range of its applications, massive and heterogeneous knowledge database, and means for content and knowledge updating and growing by its user".

The growth of the field is addressed from two perspectives: atlas construction (content, functionality, user interfaces) and use (applications and availability).

Results: Content-wise atlases develop in multiple directions: scope, parcellation, scale, modality, plurality, ethnicity, ab/normality and their combination.

The scope increases from structure to function, vasculature, connectivity, cells, molecules and genes. Spatially atlases progress from macro- to meso-, to micro-, to nano-scales. Temporal scales cover from development to lifespan. Modality-wise the development is from postmortem to in vivo data and employment of new modalities with ever-increasing resolutions. Parcellation is growing based on new modalities. Plurality encloses multiple specimens, multiple modalities and arrays of atlases. Ab/normality-wise, atlases are developed for brain in health and disease.

Application-wise, atlases are used in education (eg, diverse image sequences in www.nowinbrain.org and virtual reality), research (eg brain mapping) and clinics mainly in deep brain stimulation surgery.

There is an enormous explosion of brain-related projects leading to the construction of more advanced brain atlases: *BRAIN Initiative* creates a whole-brain cell atlas integrating molecular, anatomical and physiological types, *The Human Connectome Project* connectomics atlases and *SYNAPSE* a human brain atlas at the sub-cellular level by employing synchrotron tomography.

Conclusions: Brain atlases, aggregating the ever-growing knowledge about the brain, progress in multiple directions and are applicable in education, research and clinics.

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Session 14: Duchenne muscular dystrophy and its complications – new tools and mechanisms for possible treatments

S. 14-1. Harnessing novel 3D stem cell-based models to challenge dystrophic cardiomyopathy

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Duchenne Muscular Dystrophy (DMD) is an X-linked neuromuscular disease affecting 1:5000 live male births. This hereditary muscle disease is caused by mutations in the dystrophin gene determining progressive muscle weakness and mortality generally occurs during the second decade of life due to respiratory complications. The use of respiratory assist devices and palliative treatments has increased the life expectancy of DMD patients, thus contributing to the increase in late-stage DMD complications such as dilated cardiomyopathy. In recent years, many stem cell therapy approaches have been developed to improve muscle regeneration and to understand the role of muscle stem cell progenitors in adult myogenesis. However, no convincing results were obtained in clinical trials, supporting the idea that more fundamental research studies are necessary to better understand the DMD pathogenesis. For this, we have recently developed 3D cardiac organoid models that displayed hallmarks of progressive DMD-cardiomyopathies upon long-term dynamic cultures. These cardiac organoids have been generated from both DMD patient-iPSCs and their isogenic controls corrected via CRISPR/Cas9 gene editing technology. Despite displaying some pathophysiological relevant cytoarchitecture and functionalities, 3D cardiac organoids showed some limitations, including heterogeneity in cardiac cell sub-types that could hamper their robustness for reliable disease modelling, drug discovery, and toxicology studies. Therefore, there is a tangible need to develop more complex and reliable 3D human cell models to study DMD-related myopathies, and ultimately exploit 3D bioprinting technologies.

S. 14-2. Human induced pluripotent stem cells and gene editing for modelling of Duchenne muscular dystrophy-associated cardiomyopathy

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Duchenne muscular dystrophy (DMD) is an X-linked genetic disease caused by mutations in *DMD* gene, encoding dystrophin, which links extracellular matrix and intracellular cytoskeleton in the muscle cells. DMD patients suffer from progressive muscle dystrophy and concomitant development of dilated cardiomyopathy, currently the main cause of their premature death. The molecular basis of heart failure in DMD has not been thoroughly described due to the inaccessibility of primary cardiomyocytes from the patients and incomplete coverage of the disease progression in animal models. *Mdx* mice, for instance, the most frequently used murine model of DMD, develop relatively mild cardiomyopathy, highlighting the need to complement the animal studies with human cell-based approach.

Human induced pluripotent stem cells (hiPSC) have been extensively used to address this issue, since i) they can be generated from easily accessible somatic cells, ii) demonstrate infinite self-renewal and iii) the ability to differentiate into various cells, including cardiomyocytes (hiPSC-CM). hiPSC from different donors, however, have distinct genetic backgrounds which limits the biological validity of direct comparison between healthy and patient-specific hiPSC-derived cells for disease modeling. Thus, in our study we combined this approach with CRISPR/Cas9 gene editing which enables efficient introduction of knock-out and knock-in genetic changes into hiPSC genome. Particularly, we generated the set of isogenic control and dystrophin-deficient lines by either completely deleting *DMD* exon 50 in healthy hiPSC or by repairing *DMD* exon 48 to 50 deletion in patient-specific cells. After cardiac differentiation, a thorough characterization of such control and DMD hiPSC-CM, including transcriptomic and proteomic analyses, revealed novel druggable pathways disturbed in human dystrophin-deficient cardiomyocytes. Additionally, we have applied CRISPR/Cas9 method to knock-out and overexpress factors which can modulate the pathological alterations in DMD hiPSC-CM, providing a better way to understand their potential role in DMD-associated cardiomyopathy. Thus, our research highlights that hiPSC technology, combined with efficient gene editing, serves as a promising tool for better understanding the molecular basis of DMD progression and may facilitate the development of novel therapeutic strategies.

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S. 14-3. Hydrogen sulfide donors: therapeutic potential in Duchenne muscular dystrophy

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Background: Duchenne muscular dystrophy (DMD) is caused by mutations in the X-linked DMD gene that encodes dystrophin, a structural muscle protein. Lack of dystrophin leads to progressive muscle weakness, degeneration, and premature death, mainly due to respiratory dysfunction and cardiological complications. For now, the disease is incurable. Hydrogen sulfide (H_2S), the pleiotropic agent with antioxidant, anti-inflammatory, and proangiogenic activities, could be considered as a promising therapeutic factor for DMD.

Material and methods: To analyze the effect of H_2S on the progression of DMD, in vivo experiments have been performed using dystrophin-deficient mdx mice and their wild-type counterparts. After daily intraperitoneal administration of H_2S donors (fast-releasing sodium hydrosulfide, NaHS and slow-releasing donors: GYY4137 and AP39) for 5 weeks, functional and biochemical analysis was done on the gastrocnemius, tibialis anterior and diaphragm muscles.

Results: NaHS reduced oxidative stress and inflammation by modulating the GSH/GSSG ratio, increasing the level of cytoprotective heme oxygenase-1 (HO-1), and down-regulation of the NF-κB pathway. Furthermore, we showed a decrease in DMD biomarkers in mdx mice injected with NaHS as well as its pro-angiogenic and anti-fibrotic properties. Treatment with slow-releasing donors improved the exercise capacity and muscle strength of dystrophic animals, followed by a decrease in inflammation and muscle degeneration. Moreover, these donors reduced oxidative stress by up-regulating antioxidant proteins and cystathionine γ-lyase (CTH), the H_2S-generating enzyme. Furthermore, AMPK level was upregulated, what may affect the autophagy process.

Conclusions: These promising findings revealed the potential of new H_2S donors in attenuating the dystrophic phenotype.

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Session 15: Future therapies with psychedelics

S. 15-1. Psychedelics can produce persistent antidepressant-like effects, and changes in functional plasticity across several types of depression models

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Certain psychedelics such as psilocybin have shown profound and long-lasting therapeutic benefit in several neuropsychiatric disorders including depression and substance use disorder. Clinical trials have shown that a single treatment can have therapeutic benefits lasting months to years. The molecular mechanisms underlying these effects remain unknown, but are thought to involve changes in synaptic plasticity. We have investigated the molecular, cellular, and behavioral effects of psychedelics that may be contributing to these effects. In rat brain, we have identified specific populations of cells that transcriptionally respond to psychedelics. We have also developed rat models where a single administration of psychedelics has persistent antidepressant-like effects lasting without decline for weeks to months. We have demonstrated that even in fruit flies, a single exposure to psilocybin has antidepressant like effects. More recently, we have explored the persistence of antidepressant-like effects at the behavioral level, functional plasticity potentially underlying these effects, and have performed experiments to address whether 5-HT2A receptor activation alone is necessary for these effects. Data from each of these studies will be presented towards understanding how psychedelics may exert their therapeutic benefit in neuropsychiatric disease.

S .15-2. Psychedelics immunomodulatory effect on microglia and its prospect meaning for neurodegenerative diseases treatment

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Background: Classical psychedelics are currently undergoing their renaissance in research on prospect applications in medicine. Promising results in the treatment of major depression pointed the need to understand better the mechanisms underlying their therapeutic actions on cellular and subcellular levels. After the immunomodulatory and neurotrophic potential of psychedelics was described, the question emerged - could they also induce and regulate pro-regenerative processes in the brain and help to prevent excess neurodegeneration in the situation of neural tissue disease and damage? And since the overactive microglia accelerates the process of neurodegeneration, can psychedelic immunomodulatory properties help to prevent it?

Materials and methods: Spontaneously immortalized murine microglia cell line SIM-A9 was stimulated with 150 ng/mL LPS or FITC-labelled β-amyloid (Aβ) and treated for 24h with 1uM or 100uM of DMT and psilocin or left untreated (VEH). The inflammation related markers (IFN-γ, TNF-α, MHC-I, TLR2, CD80 and CD86), necrosis, apoptosis and proliferation KI67 markers and functional assay on Aβ phagocytosis was performed using flow cytometry

Results: The activation of SIM-A9 with LPS resulted in higher mean fluorescence intensity (MFI) of hallmark pro-inflamatory proteins (IFN-γ, TNF-α, MHC-I, CD80 and CD86) and the treatment with psychedelics resulted in their subtle immunomodulatory downregulation. The changes in MFI were especially visible in TNF-a and IFN-γ. The treatment with LPS put microglia cells into apoptosis and impaired their proliferative potential, however interestingly in the samples treated with DMT the percent of late apoptotic and dead cells were highest, whereas the treatment with psilocin resulted in the highest percent of SIM-A9 alive. The treatment with DMT and psilocin also attenuated phagocytic activity of SIM-A9 as measured with lower internalization of FITC-labelled Aβ and lower TLR2 MFI regardless of the concentration of psychedelics.

Conclusions: The data suggests that DMT and psilocin display certain potential to lower pro-inflammatory phenotype in SIM-A9 also causing attenuation in Aβ phagocytosis. For neurodegenerative disease treatment, this anti-inflammatory potential on microglia cells might be beneficial and should be further studied, however the potential risk of excess Aβ plagues formation due to attenuated phagocytosis brings serious concerns and should not be ignored.

Session 16: ERC presentation

A way to consolidate your research independence – presentation from successful ERC grant applicants

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S.16-2. Szade K.

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Session 17: Magnificent Seven: on the role of the 5-HT7R receptor in the brain

S. 17-1. Discovery of biased ligands of 5-HT7R to illuminate the receptor biology

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Transmembrane signaling through G protein-coupled receptors (GPCR), originally described as requiring coupling to intracellular G-proteins, also uses G-protein-independent pathways through β-arrestins recruitment. Biased ligands, by favoring one of the multiple bioactive conformations of GPCR, allow selective signaling through either of these pathways. This concept of functional selectivity of a ligand has emerged as an interesting property for the development of new therapeutic molecules. Biased ligands are expected to have superior efficacy and/or reduced side-effects by regulating biological functions of GPCRs in a more precise way. In the last decade, 5-HT7 receptor (5-HT7R) has become a promising target for the treatment of neuropsychiatric disorders, sleep and circadian rhythm disorders and pathological pain. We recently identified a small molecular weight compound, named Serodolin, targeting the 5-HT7R with a nanomolar affinity. We showed that Serodolin displays a baised activity : it behaves as an antagonist/inverse agonist on Gs signaling while inducing ERK activation through a β-arrestin-dependent signaling mechanism that requires c-SRC activation. Moreover, we showed that Serodolin clearly decreases hyperalgesia and pain sensation in response to inflammatory, thermal and mechanical stimulation. This anti-nociceptive effect could not be observed in 5-HT7R KO mice and was fully blocked by administration of SB269-970, a specific 5-HT7R antagonist, demonstrating the specificity of action of Serodolin. Physiological effects of 5-HT7R stimulation have been classically shown to result from Gs-dependent adenylyl cyclase activation. In this study, using a novel β-arrestin-biased agonist, we provided new insight into the molecular mechanism triggered by 5-HT7R and revealed its therapeutic potential in the modulation of pain response.

S. 17-2. Targeting the serotonin receptor 7 (5-HT7R) ameliorates Tau pathology and memory deficits

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Aggregation of the microtubule-associated protein, Tau, leads to the development multiple diseases called tauopathies, with Alzheimer's disease (AD) and frontotemporal dementia (FTD) as the most prominent members. During the last decade, the serotonergic system regained attention as a potential target for the treatment of neurodegenerative diseases, in particular AD, although the role of defined serotonin receptors in pathological Tau aggregation remained an enigma.

Our study uncovered a causal link between constitutive activity of serotonin receptor 5-HT7 (5-HT7R) and pathological Tau hyperphosphorylation and the formation of neurofibrillary tangles in primary neuronal cultures and in cortical neurons in vivo. We elucidated the underlying molecular machinery by demonstrating a physical interaction between 5-HT7R and the Tau kinase, CDK5, leading to a G protein independent activation of CDK5. We also defined the structural requirements for the 5-HT7R and CDK5 interaction and deciphered 5-HT7R/CDK5 interaction interface.

The therapeutic potential of the 5-HT7R/CDK5 pathway was demonstrated by showing that the selective knockdown of the 5-HT7R in the prefrontal cortex (PFC) of mice abrogated the deleterious effects of Tau[R406W] overexpression in this region on synaptic plasticity and cognition. Using structural and functional screenings, we identified several clinically approved drugs to possess a high inverse agonism towards the 5-HT7R, with amisulpride being more prominent candidate. Its therapeutic potential was validated using biochemical, pharmacological, microscopic and behavioral approaches in different cellular models including primary mouse neurons and human iPSC-derived neurons carrying a FTD-associated Tau mutation as well as in two mouse models of tauopathy. Treatment with amisulpride ameliorated various aspects of Tau pathology, including abrogation of hyperphosphorylation, tangle formation, apoptosis, and memory deficits in a mouse model of tauopathy.

Taken together, our findings demonstrate that pharmacological targeting of 5-HT7R by inverse agonists represents a new strategy for the treatment of tauopathies.

S. 17-3. The molecular fingerprint of stress resilience

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Stress resilience is an ability of neuronal networks to maintain their function despite the stress exposure. In this study, we investigate whether stress resilience is an actively developed dynamic process in adult mice. In order to assess the resilient and anhedonic behavioral phenotypes developed after induction of the chronic unpredictable stress, we quantitatively characterized the structural and functional plasticity of excitatory synapses in the hippocampus using a combination of proteomic, electrophysiological, and imaging methods. Our results indicate that stress resilience is a dynamic and multifactorial process manifested by structural, functional, and molecular changes in synapses. We reveal that chronic stress influences palmitoylation, whose profiles differ between resilient and anhedonic animals. We also observed that stress resilience is associated with structural compensatory plasticity of the postsynaptic parts of synapses.

Session 18: Young Scientific investigators session

S.18-1. Presence of HCAR3 in human skin in psoriasis

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Background: Psoriasis is a chronic, noncommunicable, stigmatizing skin disease that is estimated to affect 60 million people worldwide. The etiology of psoriasis remains unknown. The disease so far is incurable. Subjects require treatment to alleviate symptoms or cause remission usually lifelong. Therefore, there is a constant need to search for the disease's pathogenesis and new therapeutic targets.

Over the past decade, tryptophan metabolites formed along kynurenine pathway, among them kynurenic acid (KYNA), have attracted considerable interest. KYNA is an endogenous substance with anti-inflammatory, antioxidant and pain modulating effects. Its beneficial wound healing properties were described. Importantly, KYNA was found in human sweat. Its significance in psoriasis is completely unknown although there is much interest in its effects exerted on glutamate receptors, G protein-coupled *receptor* 35 and aryl hydrocarbon receptor. Recently it was discovered that KYNA is a ligand of G protein-coupled hydroxycarboxylic acid *receptor* 3 (HCAR3).

Aim: The aim of the study was to investigate whether the HCAR3 receptor occurs in the skin and what is its location in human skin sampled from the psoriatic lesion area.

Materials and methods: The human skin was collected during the routine diagnostic procedure. The paraffin block was obtained courtesy of the owners of the ALFAMED company (Ćwierz Edward MD, Ćwierz Maciej MSc). Psoriatic tissue was subjected to immunohistochemical analysis of HCAR3 (polyclonal, Cell Signaling Technology, 1:100).

Results: HCAR3 receptor was identified in all layers of the epidermis, mainly with a cytoplasmic, less with a membrane reaction. HCAR3 was observed in the heterogeneous elongation of epidermal icicles (with histological changes characteristic of psoriasis like acanthosis and hyperplasia) strongly in keratinocyte with impaired differentiation and keratinization. High expression of HCAR3 was presented in the thickened stratum corneum, partially compacted, with features of focal parakeratosis. In neutrophils within Munro's microabscesses we didn't observed a positive staining reaction.

Conclusions: The presence and specific localization of HCA3 in psoriasis-affected skin provides a rationale for further exploration of the role of this receptor in pathogenesis of the disease and points to potential new therapeutic target in psoriasis.

Acknowledgements: The paraffin block was obtained courtesy of the owners of the ALFAMED company.

S.18-2. Targeting SNAIL in rhabdomyosarcoma: antisense oligonucleotides and miRNA as promising agents for future therapies

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Background: SNAI1 is a transcription factor that regulates E-cadherin repression during the epithelial-mesenchymal transition (EMT). Dysregulation of SNAIL expression has been shown to be associated with the development and progression of various tumours, including rhabdomyosarcoma. Current therapies for this disease remain suboptimal, highlighting the need for novel therapeutic strategies. Our previous investigations have shown that targeting SNAIL in rhabdomyosarcoma may be a promising approach for future therapies.

Antisense oligonucleotides (ASOs) have gained attention for their potential use as a targeted therapy against disease-associated genes. Their mechanism of action offers potential advantages over traditional small-molecule drugs and monoclonal antibodies, making them an attractive option for innovative treatment strategies. MicroRNAs (miRNAs) are small, non-coding RNA molecules that regulate gene expression by binding to target mRNAs. Among the miRNAs that have been implicated in EMT and tumorigenesis is the miR-30 family, which has been shown to negatively regulate SNAI1 expression.

The present study aims to explore the potential of miR-30a and ASOs targeting SNAIL as therapeutic agents for rhabdomyosarcoma.

Material and methods: We designed ASOs targeting SNAIL and a cholesterol-conjugated miR-30a. To assess their therapeutic potential, we evaluated the ability of ASO and miR-30a to down-regulate SNAI1 expression and inhibit the proliferation of rhabdomyosarcoma cells (Rh30 cell line). We also examined the effectiveness of miR-30a uptake by measuring intracellular miR-30a levels. Finally, the intracellular stability and retention of the SNAIL silencing agents were evaluated to assess sustained therapeutic efficacy.

Results: We demonstrated high efficiency of SNAIL targeting using designed ASOs, which strongly impacted cell viability. The miRNA experiments showed that Chol-miR-30a exhibited twice as high uptake efficiency compared to unconjugated miR-30a and was more effective in down-regulating SNAI1 expression in rhabdomyosarcoma cells.

Conclusions: In conclusion, our findings provide valuable insight into the potential of ASO and miR-30a as a novel therapeutic approach for rhabdomyosarcoma treatment and facilitate the development of lipoprotein-conjugated miRNA delivery systems for other types of cancer.

Acknowledgements: This work was supported by research grant 2018/29/B/NZ5/00915 from the National Science Center in Poland.

S.18-3. Efficient CRISPR/Cas9-mediated correction of *DMD* exon deletion in Becker Muscular Dystrophy patient-derived induced pluripotent stem cells

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Background: Becker muscular dystrophy (BMD) is an X-linked recessive disorder caused by in-frame mutations in the *DMD* gene encoding dystrophin. Cardiomyopathy is the leading cause of death in this disease. However, the mechanisms of BMD are difficult to investigate due to the scarcity of animal models as well as human samples. Fortunately, induced pluripotent stem cells (iPSCs) represent a novel tool for BMD modeling, preserving the patient's genetic background. Therefore, the aim of this study was to generate patient-specific BMD iPSCs expressing shortened dystrophin and relevant isogenic lines with repaired *DMD* exon deletions by CRISPR/Cas9 gene editing.

Material and methods: Three BMD patients with an in-frame deletion of exons: 3 to 4, 3 to 9, and 45 to 47 were involved in the study. Patient-specific BMD iPSCs were generated from peripheral blood mononuclear cells using Sendai vectors delivering reprogramming Oct4, Sox2, Klf4, and c-Myc genes. Nucleofection was then used to introduce plasmids encoding a single guide RNA (sgRNA) targeting the mutated *DMD* region and a repair template containing homologous arms missing *DMD* exons into these cells. Following the differentiation of the cells into cardiomyocytes (iPSC-CM), RT-PCR and Western Blot analysis were performed to confirm the knock-in of missing exons.

Results: Successful generation of BMD iPSC lines was first confirmed by demonstrating the expression of pluripotency markers, the ability to differentiate into the derivatives of three germ layers as well as the lack of *DMD* exons underlying BMD development. Subsequently, two plasmid variants encoding distinct sgRNAs as well as Cas9 were created. The Surveyor nuclease assay selected the most efficient of the two designed sgRNAs and the corresponding plasmid together with repair templated were introduced into the iPSCs. Obtained cells were differentiated into iPSC-CM after clonal selection and analysis. The mutation repair was finally confirmed, demonstrating the presence of full-length dystrophin protein in the cells.

Conclusions: CRISPR/Cas9-mediated genome editing has been shown to successfully correct genomic mutations in iPSCs derived from BMD patients.

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S.18-4. Model Preparation and Virtual Screening for Chemokine Receptor CXCR3

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Background: Chemokines are small proteins that regulate the migration of immune cells and are responsible for the modulation of the immune response. Inflammatory chemokines, specifically, facilitate the chemotaxis of leukocytes to injured or inflamed areas of the body. These effects are expressed through the activation of chemokine receptors, which can be divided into four different groups depending on which chemokine subfamilies they bind: CCR, CXCR, XCR, and CX3CR. Chemokine receptors have been linked to numerous diseases—for example, CXCR3 ligands are known to play a role in the immune response to e.g., chronic hepatitis C virus infection, the West Nile virus, and the Dengue Virus. Despite this, there are not yet any drugs targeting this receptor. Drug discovery may be hindered by the fact that there are no available crystal structures of the receptor—this issue can be circumvented by using homology modeling or threading to create receptor models to serve as a basis for structure-based virtual screening (SBVS).

Materials and Methods: Modeller was used to create models of CXCR3. Their energy-based scores were assessed and structures analyzed in order to select the best quality model. LBVS supported by machine learning (Dragan et al. Pharmaceutics, 2023) was followed by SBVS performed using AutoDock Vina on an Enamine compound library.

Results: The best CXCR3 models were discussed in terms of residues that could be involved in the ligand binding and in terms of molecular switches important for the receptor activation. 10 best-scoring compounds were selected as scaffolds for further drug design. Possible chemical modifications of these compounds were proposed to obtain novel CXCR3 actives.

Conclusions: Chemokine receptors constitute promising drug targets due to their role in numerous inflammatory processes. CXCR3 is one such example and the creation of models for this receptor of a yet unknown structure can facilitate structure-based virtual screening, which itself is a useful tool for decreasing the number of possible drug scaffolds.

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S.18-5. PZ-2172, a potent, selective and metabolically stable 5-HT7 receptor inverse agonist with antidepressant-like and procognitive properties in rodents

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Background: 5-HT_7 receptor (5-HT_7R) represents the latest addition to a subfamily of serotonin receptors. Apart from the coupling to adenylyl cyclase through G_s protein (canonical) and G_{12} (non-canonical), 5-HT_7R has recently been shown to engage G-protein-independent signaling pathways (e.g., β-arrestin). 5-HT_7R has been considered as novel therapeutic target for the treatment of depression and co-morbid cognitive dysfunctions. Continuing our effort on the development of 5-HT_7R antagonists, a series of novel derivatives has been designed, synthesized and biologically evaluated *in vitro* identifying compound **PZ-2172** as a lead structure.
Materials and methods: The affinity of **PZ-2172** for 5-HT_7R and selectivity over related serotonin and dopaminergic receptors ($5HT_{1A}$, 5-HT_{2A}, 5-HT_6 and D_2Rs) were determined by radioligand binding experiments in HEK2-93 cells. The effect on Gs-operated 5-HT_7R constitutive activity was evaluated using their ability to inhibit cAMP production induced by agonist 5-CT (10 nM) in a HEK-293 cells. The metabolic stability of **PZ-2172** was assessed in rat liver microsomes (RLM) assay while its preliminary safety profile was evaluated in HepG2 cells using the MTT test. Evaluation of the pharmacokinetic profile after *i.g.* administration of a dose of 3 mg/kg, was performed in Wistar rats. Antidepressant and pro-cognitive properties were examined in *in vivo* in tail suspension test (TST) and novel object recognition task (NORT), respectively.
Results: **PZ-2172** behaves as potent 5-HT_7R inverse agonist at G_s signaling pathway (K_i 5-HT_7 = 3 nM, IC_{50} = 3.2 nM), displaying high selectivity over tested GPCRs (selectivity index > 130). Compound **PZ-2172** is characterized by good *in vitro* metabolic stability, high *in vivo* oral bioavailability, well brain-penetration and low hepatotoxic effect. **PZ-2172** exerted antidepressant-like activity and pro-cognitive properties at the dose of 0.625 and 1 mg/kg (*i.p*), respectively. Of note, the potency was similar to that of the reference SB-269970.
Conclusions: The promising pharmacological properties *in vitro* and *in vivo*, along with favourable drug-likeness profile, justify further development of **PZ-2172** as a molecular probe to confirm the role of 5-HT_7 receptor in affective disorders.
Acknowledgements: This study was supported by the National Science Centre, Poland (grant no. 2019/33/B/NZ7/02822).

POSTER SESSIONS I

Preclinical Research

P.1-1. Generation of human induced pluripotent stem cells for modeling of Spinal Muscular Atrophy and Amyotrophic Lateral Sclerosis

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Background: Disease modelling of neuromuscular disorders is hindered by limited accessibility of primary motor neurons, both from the patients and healthy controls. Human induced pluripotent stem cells (hiPSC) have emerged as unique tool to address this issue as they can be generated from easily accessible somatic cells and differentiate into myogenic and neuronal lineages. These cells are particularly useful for modelling of diseases with known genetic background. Thus, the aim of this study was to derive hiPSC lines from patients with spinal muscle atrophy (SMA), caused by mutations in SMN1 and amyotrophic lateral sclerosis (ALS) driven by G4C2 expansion in C9ORF72 gene.

Methods and Results: Peripheral blood mononuclear cells were isolated from three SMA and two ALS patients and further transduced with non-integrating Sendai vectors encoding OCT4, KLF4, SOX2 and c-MYC. Successful reprogramming was confirmed by demonstrating expression of pluripotency markers: OCT4, NANOG, SSEA4, TRA-1-60 and TRA-1-81 in obtained hiPSC lines and their ability to differentiate into cells originating from three germ layers. In the next step, CRISPR/Cas9 strategy was designed to repair disease-triggering mutations in patient-specific hiPSC. Particularly, in case of SMA, a single-guide RNA (sgRNA) targeting AAVS1 locus, located in chromosome 19, together with the template containing homologous arms and SMN1 coding sequence, were delivered into hiPSC line from one patient. Successful gene editing and the restoration of SMN1 expression was then confirmed with qPCR in selected clones. Isogenic control cells were prepared in the same manner using repair template without SMN1 coding sequence. In case of ALS, another strategy was applied based on two sgRNA molecules targeting the flanking regions of G4C2 expansion located in the intron 1 of C9ORF72 enabling complete removal of this sequence. Additionally, a repair template containing homologous arms and non-mutated version of targeted locus was constructed and delivered into hiPSC line from one patient together with sgRNA-encoding plasmids.

Conclusions: SMA and ALS patient-specific hiPSC lines have been successfully generated together with the respective isogenic repaired lines, a human cell-based system for deciphering molecular basis of these devastating neuromuscular diseases.

Grants support: National Science Centre: JPND grant [UMO-2019/01/Y/NZ3/00012].

P.1-2. mGluR2 negative allosteric modulator VU6001966 enhances scopolamine-induced antidepressant-like effects in the UCMS model of depression

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Background: Conventional antidepressants typically require weeks of daily dosing to achieve a full antidepressant response. In contrast, clinical studies have demonstrated that scopolamine, a non-selective antagonist for muscarinic receptors, can induce potent and rapid antidepressant effects after just a few days of treatment. However, the side effects of scopolamine are still a matter of concern. To address these challenges, our study focused on exploring the potential antidepressant activity of a sub-effective dose of scopolamine administered jointly with a sub-effective dose of $mGlu_2$ negative allosteric modulator (NAM) VU6001966 in unpredictable chronic mild stress (UCMS) model of depression. Additionally, preliminary in vitro studies were conducted to evaluate the pharmacological modulation of both $mGlu_2$ and selected muscarinic receptors expressed in the T-REx 293 cell line by using a cAMP accumulation assay.

Material and methods: Unpredictable chronic mild stress model of depression was established in male C57BL/6J mice. The animals underwent subchronic treatment for four consecutive days, receiving either scopolamine (0.1 mg/kg), VU6001966 (1 mg/kg), or both in combination. Then, 24 hours after the last treatment, two parameters reflecting the core symptoms of depression were analyzed: reduced grooming time in the splash test, which reflects apathy, and decreased sucrose preference, which indicates anhedonia. cAMP accumulation assay was conducted by using the T-REx 293 cell line with tetracycline-based inducible expression of $mGlu_2$ and stable expression of the selected muscarinic receptor.

Results: We have found that the combination of scopolamine (0.1 mg/kg) and VU6001966 (1 mg/kg), administered for four consecutive days, significantly reversed the UCMS-induced depressive-like behaviors, such as apathy and anhedonia. Furthermore, our preliminary in vitro studies can suggest the existence of interactions between $mGlu_2$ and selected muscarinic receptors.

Conclusions: Our results show that the coadministration of scopolamine with $mGlu_2$ NAM might be a noteworthy alternative to the use of scopolamine alone in the therapy of depression, allowing its therapeutically effective dose to be lowered.

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P.1-3. Comparative study on the effects of chronic treatment with the atypical antipsychotic drug lurasidone on cytochrome P450 enzymes in rat liver and lymphocytes

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Background: Lurasidone is an atypical antipsychotic drug acting on dopaminergic, serotonergic and noradrenergic receptors, applied for the long-term treatment of schizophrenia and depression in patients with bipolar disorders. Lurasidone is primarily metabolized in the human liver by CYP3A4. The aim of the work was to perform a comparative study on the *in vivo* effect of two-week treatment with lurasidone on the expression of cytochrome P450 enzymes in the liver and in peripheral blood lymphocytes, and to evaluate relationship between changes in the expression of CYP enzymes in those two experimental models.

Material and methods: Male Wistar Han rats were administrated lurasidone (1mg/kg ip.) for 2 weeks. Afterwards, livers were isolated and lymphocyte cells were collected. The activity of CYP enzymes in liver microsomes was estimated by measurement of the rate of CYP specific reactions (HPLC with UV or fluorometric detection). The CYP protein levels were assessed by Western Blot. The expression of *CYP* genes (mRNA levels) was determined by qRT-PCR. The results were elaborated statistically using Student's t-test.

Results: The obtained results show quite similar pattern of main CYP enzymes' expression in the rat liver and lymphocytes. They indicate that in the liver lurasidone exerts an inhibitory effect on the activity, protein and mRNA level of CYP2B1/2 (not *CYP2B2* mRNA), CYP2C11 and CYP2E1, while in the case of CYP3A1 and CYP3A2 it causes enzyme induction. At the same time, lurasidone decreases the expression of CYP2B, CYP2C11 (CYP2C11 protein only) and CYP2E1, but increases that of CYP3A2 (not CYP3A1) in lymphocyte cells.

Conclusion: The present study provide evidence that chronic treatment with lurasidone simultaneously and in the same direction influences the expression and activity of CYP2B, CYP2C11, CYP2E1 and CYP3A in the rat liver and peripheral blood lymphocytes. Thus, the lymphocyte cytochrome P450 profile may be employed as an indicator of the hepatic cytochrome P450 profile in further studies and may serve as an easily accessible surrogates for investigating the impact of new drugs and chronic treatments on CYP enzyme expression, and to estimate drug-drug interaction and toxicity risk.

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P.1-4. The effects of histamine H_3 receptor antagonist, pitolisant, on activation of cultured murine glial cells

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Background: Neuroinflammation plays a key role in the pathophysiology of many neurological disorders. Glial cells, especially microglia and astrocytes, are specialized in immune functions and protect the brain tissue. Within the CNS, histamine has been shown to critically modulate inflammatory processes. Its physiological functions are mediated by four receptors ($H_{1-4}R$) that are all targets of pharmacological intervention. We hypothesized that histamine H_3R antagonists might play a role in glia activation and related neuroinflammation. The aim of our study was to evaluate the effects of Pitolisant (PIT), an H_3R antagonist, on inflammatory markers in cultured murine glial cells exposed to lipopolysaccharide (LPS).

Materials and Methods: In murine microglial BV-2 cell line the effects of PIT (10, 1, 0.1, 0.01 μM) on cell viability and nitric oxide (NO) production were determined by MTT and Griess assays, respectively. The influence of PIT (10 μM) treatment on cytokines production was assessed in primary mouse microglial and astrocyte cell cultures prepared from cortices of postnatal mouse pups. The presence of H_3R in primary glial cells was confirmed by immunocytochemistry. PIT was added 48 and 72 h after seeding of microglia and astrocytes, respectively, 30 min prior LPS (100 ng/mL). Cell media and/or lysates were collected 24 h following stimulation. Protein levels of IL-1β, IL-6, IL-10 were quantified by ELISA. Changes in the expression of microglia cell markers, CD206 and Iba1, were examined by Western blot.

Results: PIT did not affect BV2 cells viability nor NO production. Immunocytochemical staining confirmed the presence of H_3R on primary murine microglial and astroglial cells. We did not observe any changes in cytokine levels after PIT treatment in primary microglia. However, it significantly increased IL-1β while at the same suppressing IL-10 production in primary astrocytes. Western blot analysis showed decreased microglia activation marker Iba1 after PIT stimulation while CD206 level remained unchanged.

Conclusions: Our results indicate that PIT modulates glial-induced neuroinflammation, mainly affecting astroglial cells. The results of our studies represent an important area of future research as modulation of glia-induced neuroinflammation by H_3R antagonist may provide novel therapeutic targets in many CNS disorders.

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P.1-5. Targeted heme oxygenase-1 expression promotes satellite cell proliferation and modulates muscle pathology in dystrophic mice

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Skeletal muscle stem cells, satellite cells (SCs), that normally play essential and non-redundant role in skeletal muscle regeneration, are intrinsically impaired in Duchenne muscular dystrophy (DMD). Dystrophic SCs express low level of anti-inflammatory and anti-oxidative heme oxygenase-1 (HO-1, *HMOX1*). We aimed to determine whether targeted induction of *HMOX1* in SCs contributes to the muscle phenotype in dystrophic (*mdx)* mice.

Although high intensity treadmill exercise increased SC activation, it significantly decreased the percentage of SCs ($CD45^-/CD31^-/Sca\text{-}1^-/\alpha7integrin^+$) in hind limb skeletal muscles. Tamoxifen (TX)-induced SC-targeted *HMOX1* expression in dystrophic mice (*mdx*-Flox-HO-1) decreased SC pool without affecting SC activation. Upon physical exercise, the percent of proliferating SCs decreased in control *mdx* mice, an effect that was reversed in the presence of HMOX1 expression. Such run-induced changes corresponded to the pattern of murine *Hmox1* expression in respective genotypes. Moreover, physical exercise-induced necrosis of selective skeletal muscle and associated increase in circulating levels of muscle damage markers was blunted in the animals that expressed *HMOX1*. Furthermore, *HMOX1* expression was associated with an increase in anti-inflammatory cytokine, IL-10 and an increase in myogenic markers, MyoD and MyoG.

Taken together, our study reveals the stimulatory effect of SC-targeted *HMOX1* expression on SC proliferation and associated protection against basal- and running-related necrotic events in selective skeletal muscles of dystrophic mice.

P.1-6. Dysfunction of liver cytochrome P450 enzymes associated with aging and the effect of brain serotonin deficit on their regulation in senescent rats

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Background: Liver cytochrome P450 (CYP) plays an important role in the metabolism of endogenous substances and xenobiotics (drugs, carcinogens and toxins). Recent studies in rodents have demonstrated that CYP expression in the liver is controlled by the central nervous system *via* hormonal pathways. In particular, the expression of hepatic CYPs is negatively regulated by the brain serotoninergic system. The aim of the study was to investigate changes in the function of liver CYP enzymes in rats during aging and as a result of serotonin deprivation in the brain.

Material and methods: Male Dark Agouti rats of the wild type (adult and senescent) and genetically modified animals lacking *the tryptophan hydroxylase 2 gene (TPH2-KO)* served as experimental animals. Major CYP enzymes (CYP2C11, CYP3A1/2, CYP2C6, CYP2B1/2, CYP2A1/2, CYP1A1/2) were examined in rat liver microsomes by measuring 1) mRNA levels of *CYP* genes (qRT-PCR); 2) CYP protein levels (Western immunoblot analyses); and 3) CYP enzyme activities (based on metabolic reactions specific for individual CYP enzymes, using HPLC with UV or fluorescence detection). Student's t-test was used for the statistical analysis of results.

Results: In general, the expression and activities of the CYP enzymes studied were reduced in aging animals compared to adults. In aging TPH2-deficient males, the expression of CYP2C11 and CYP3A decreased in the liver, while the activity of these enzymes increased relative to aging wild-type males. At the same time, the *TPH2-KO* mutation tended to enhance the expression and activity of the CYP2C6 enzyme.

Conclusions: The results of studies into expression and activity of cytochrome P450 in senescent rats show that hepatic CYP enzymes of male rats function less effectively with age, which may impair drug metabolism in the liver. In addition, the results indicate that brain serotonin is involved in the regulation of hepatic cytochrome P450 expression and activity in male rats.

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P.1-7. Polymeric -based nanocarriers for delivery of neuroprotective drugs though blood-brain barrier

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Background: The age-dependent neurodegenerative diseases are becoming increasingly prevalent in rapid aging of populations around the world. One of the major limitations in treatments of neurodegenerative diseases is an inefficient delivery of neuroprotective drugs through the blood-brain barrier (BBB) that is permeable only to small, lipophilic molecules. The very poor water solubility of some neuroprotectants limits their delivery to the affected part of the brain. Moreover, the targeted and monitored delivery of neuroprotectants in nanocapsules enables avoiding peripheral undesired effects and ensures obtaining their therapeutic concentrations in the specific area of the central nervous system.

Material and methods: We present a novel methodology for delivering selected neuroprotective substances through BBB using multifunctional nanocarriers based on polymeric nanoparticles (NPs). For initial tests two types of empty nanocarriers abbreviated as AOT/(PLL/PGA)2-g-PEG and PCL/(PLL/PGA)2-g-PEG loaded with rhodamine as fluorescent marker were selected. Due to the fact that the hCMEC/D3 human cell line, was shown to retain important BBB characteristics, the abovementioned model was chosen as suitable for testing BBB permeability for putative nanocarriers. The hCMEC/D3 cell line was grown in supplemented EBM-2 medium. Cells were cultured at optimal density on transwell filters (24-well plates) for 5 days to form confluent monolayer. Trans Epithelial Electrical Resistance (TEER) of epithelial cells were checked every day. The assay was performed 5 days after seeding. Rhodamine-labeled nanoparticles at different concentrations were added to apical chamber. The basolateral chamber consisted of fresh FluoroBrite medium without NPs. A fluorescent signal was measured in the basolateral chamber at various exposure times to nanocarriers.

Results: The results clearly indicated that both types of NCs were able to cross BBB. Time-dependent changes in fluorescence intensity of medium from lower compartment showed that the maximum signal was obtained after 48 hour exposure. The maximum fluorescence intensity corresponded to the maximum quantity of NCs that passed BBB in this in vitro model. Conclusions: Presented data are promising, however further studies which may provide insights to improve treatment and therapies of neurodegenerative diseases are needed.

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P.1-8. Transgenerational effects of maternal high-fat diet on epigenetic and morphological changes within the prefrontal cortex in rat offspring

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Background: Recent preclinical and clinical findings suggest a strong relationship between maternal obesity and the risk for autism spectrum disorder (ASD) in offspring. ASD is a complex neurodevelopmental disorder whose prevalence has increased rapidly over the last few decades. The continuous increase in the incidence of ASD has intensified research on the role of epigenetic factors (mainly abnormal DNA methylation) and environmental interactions. The present study aimed to determine the effects of a maternal high-fat diet (HFD) during pregnancy and lactation on transgenerational epigenetic changes in the offspring's prefrontal cortex.

Material and methods: Wistar rat dams were fed either a standard diet or an HFD during pregnancy and lactation. After weaning, offspring were separated according to sex and fed an SD. A subset of F1 females was used to create the F2 generation. For molecular analysis, at postnatal day 28, male and female offspring from generations F1 and F2 were sacrificed, and the prefrontal cortex was dissected. Next, the DNA methylation (5-mC) and hydroxymethylation (5-hmC) were assessed using ELISA kits. Moreover, the expression of parvalbumin-positive (PV+) cells in the prefrontal cortex was evaluated using immunofluorescence staining and confocal microscopy.

Results: In generation F1, a significant increase in global DNA methylation (5-mC) and hydroxymethylation (5-hmC) was observed only in male offspring. The increased levels of global 5-mC and 5-hmC were also reported in males from generation F2, while female offspring from generation F2 had decreased prefrontal levels of 5-mC. Additionally, the assessment of interneuron expression showed that a maternal HFD reduced the number of PV+ cells in the prefrontal cortex of male offspring from the F1 generation, which may contribute to an imbalance between excitatory and inhibitory signaling.

Conclusions: To sum up, the obtained results confirm the transgenerational influence of the intrauterine and early childhood environment on proper brain development, especially in the male F1 generation. Moreover, these observations may partially explain the gender differences noted in our previous behavioral studies where we pointed out that only male offspring exposed to a maternal HFD showed an autistic-like phenotype.

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P.1-9. Prenatal dexamethasone treatment increases the level of lactate but reduces its transport from astrocytes to neurons in the frontal cortex of rats

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Background: Lactate, the end product of glycolysis, belongs to the family of monocarboxylates which requires the presence of transporters to cross biological membranes. Monocarboxylate transporter 4 (MCT4) is responsible for the efflux of lactate from astrocytes, while monocarboxylate transporter 2 (MCT2) is involved in the influx of lactate to neurons. Lactate is not only an important source of energy but also a multifunctional signaling molecule at cellular, tissue, and organ levels. What is important, both deficiency and accumulation of lactate can cause neuropsychiatric disorders associated with mitochondrial dysfunction and among them – depression.
Materials and methods: The study was conducted in the animal model of depression. Pregnant Sprague-Dawley rats were treated with synthetic glucocorticoid (0.1 mg/kg dexamethasone, DEX) starting from the 14^{th} day of pregnancy until delivery. Adult male offspring of control and DEX-treated dams underwent behavioral verification and the additional subdivision of these two groups was done. Two hours before being sacrificed, subgroups of the DEX-treated and control animals were subjected to a single session of acute immobilization stress. Frontal cortices were dissected for subsequent biochemical analysis with the use of a colorimetric method and immunoblotting. The results were analyzed using the STATISTICA software. A p-value < 0.05 was considered to be significant.
Results: In the cytosolic fraction of the frontal cortex, lactate levels were found to be elevated by DEX and/or additional stress in all experimental groups. Moreover, in animals prenatally exposed to DEX and subjected to acute stress in adulthood the levels of both lactate transporters, i.e., MCT2 and MCT4 were diminished.
Conclusions: In conclusion, in the animal model of depression based on prenatal DEX administration, disturbances in lactate production and its transport between the cells were demonstrated in the frontal cortex – one of the brain structures most consistently impaired in depression. Observed changes may affect neuronal energy metabolism and result in abnormal brain activity but further studies are required to confirm this hypothesis.
Acknowledgments: This work was supported by grant no. 2020/39/D/NZ7/01610 from National Science Centre, Poland.

P.1-10. Optimalization of the tight junctions' investigation procedure in Caco-2 cells with use of RTCA S16 device

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Background: The cellular-based impedance assay (IBCA) is a novel, non-invasive, label-free method that measures impedance of cells in real-time. The quantitative growth measurement is based on the readouts of the electrical impedance at the gold-plated electrodes in microplate having 16 wells (E16 xCELLigence). The experiment was run on the D cradle of the real time cell analyzer (RTCA) instrument. The cell line used was Caco-2 which is derived from human colorectal adenocarcinoma (ATCC® HTB-37). It is a suitable model which resembles intestinal epithelial cells as they form a polarized and continuous monolayer of cells that expresses tight junctions, microvilli, transporters, barriers, and adhesions on reaching confluency in 18-20 days.

Material and methods: Experiments were performed using different densities of cells including. After reaching confluency 80%-90%, the cells were freshly split, counted, and resuspended in complete culture media. To monitor proliferation the cells were added equally per row in all wells along with blank (cells free media) wells. The time between sweeps was set for 1 or 3 hr for the measurement of any change in the proliferation, adherence, morphology, and viability, and total number of sweeps were set for 160. The cell index (CI) was measured based on relative change in electrical impedance. The RTCA instrument monitored cell growth and proliferation for up to 400 hours. After the monolayer formation, the reference compound TNF-α was added in the concentration range 0.1-100 ng/ml and then incubated for 72 hr for real-time observation of its effect on TJs in Caco-2 cells.

Results: The CI goes up with an increase in cells' adhesion and number, the CI flattens at full confluency, and it decreases when cells die. We found that the magnitude of impedance depends on the number of cells seeded at the beginning of the experiment, concentration of FBS in media as well as on the number of media exchanges. Thus, the proper parameters for cells grow and investigation of TJs in Caco-2 cells were selected. The effect of TNF-α of TJs was also successfully monitored in real-time.

Conclusions: Our results conclude that real-time cellular biosensor is a valuable platform and alternative for TEER method for measurements of quantitative proliferation curves of Caco-2 cells and the identification of quantitative cells' response on the tested compounds including the effect on TJs.

P.1-11. Effect of treatment with antidepressants and L-DOPA on radioligand binding to monoamine transporters in the nucleus accumbens of 6-OHDA-lesioned rats.

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Background: Antidepressants are recommended in the treatment of depression accompanying Parkinson's disease, however, in the symptomatic phase of the disease, their administration in combination with L-DOPA also affects motor disorders. The aim of the study was to investigate the effects of chronic treatment with antidepressants, such as amitriptyline (AMI), fluoxetine (FLU) and the antiparkinsonian drug L-DOPA, on binding of radioligands to monoamine transporters in the nucleus accumbens (NAc) of unilaterally 6-OHDA-lesioned rats.

Material and methods: Experiments were performed on rats that were injected unilaterally with 16 µg/4µl of 6-OHDA to the medial forebrain bundle. Two weeks after lesion, rats exhibiting an extensive loss of nigrostriatal neurons received AMI (10 mg/kg), FLU (5 mg/kg) or L-DOPA (12 mg/kg), alone or in combination, once daily for 21 days. After the last drug injections, the rats were sacrificed and their brains were dissected and frozen. The binding of [^{3}H]GBR 12,935 to dopamine transporter (DAT), [^{3}H]citalopram to serotonin transporter (SERT) and [^{3}H]nisoxetine to noradrenaline transporter (NET) was assayed in NAc (core and shell) tissue sections.

Results: Unilateral injection of 6-OHDA into MFB resulted in a drastic decline in [^{3}H]GBR 12,935 binding to DAT in both the core and shell of the NAc on the ipsilateral side, while an increase in this binding was observed on the contralateral side. [^{3}H]citalopram binding to SERT in the lesioned rats remained unchanged in the ipsilateral NAc, but increased significantly on the contralateral side. In the lesioned rats, [3H]nisoxetine binding to NET was significantly increased in both the ipsilateral and contralateral NAc. Chronic L-DOPA treatment did not alter [^{3}H]GBR 12,935 binding to DAT in the ipsilateral NAc, but binding of [^{3}H]nisoxetine to NET decreased in both the ipsilateral and contralateral NAc. Both AMI and FLU, alone or in combination with L-DOPA, significantly reduced [^{3}H]citalopram binding to SERT in the ipsilateral and contralateral NAc. Co-administration of AMI or FLU with L-DOPA restored [3H]nisoxetine binding to NET to control levels.

Conclusions: The obtained results suggest that both AMI and FLU modulate the SERT function in the NAc, which may have functional implications.

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P.1-12. Acute administration of psilocybin alters gene expression within the lateral habenula in WKY rats

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Background: Treatment-resistant depression (TRD) is a challenging condition experienced by approximately 30% of individuals with depression. Due to the high number of treatment failures, new therapeutic compounds are constantly sought. Psilocybin, a serotonergic psychedelic found in "magic mushrooms", is suggested to have therapeutic potential for conditions such as treatment-resistant depression, anxiety, obsessive-compulsive disorder, and addiction.

Wistar Kyoto (WKY) rats are considered a suitable animal model for studying treatment – resistant depression in preclinical research due to their significant similarities to the human condition. The administration of psilocybin to WKY rats allowed to investigate the molecular alterations occurring within the lateral habenula (LHb), a critical region implicated in drug-resistant depression.

Material and methods: Brain tissue was collected from male Wistar and WKY rats four hours after an acute intraperitoneal injection of 0.3 mg/kg psilocybin. Laser Microdissection was used to isolate the LHb. Following RNA isolation, gene expression levels were evaluated using TaqMan Array Cards, which contained 32 depression-related genes selected based on literature data.

Results: Following psilocybin injection, downregulation was observed in two genes in WKY rats: *Kcnj5* and *Htr7*. In contrast, psilocybin did not significantly alter the expression of these genes in Wistar rats. Furthermore, strain effects were observed for several genes: *Cacna1b*, *Cdkn1c, Htr2a*, *Mapk14*, *Slc12a5*, *Slc6a6*, *Sstr2*, and *Sstr4.* Additionally, an interaction strain x psilocybin was observed for *Mapk14* and *Tph1* genes.

Conclusions: The study aimed to investigate the molecular alterations occurring in the LHb of WKY rats after psilocybin treatment. The findings revealed notable effects of psilocybin on gene expression in WKY rats. Specifically, *Kcnj5* and *Htr7* exhibited downregulation, suggesting that psilocybin may have a specific impact on these genes in the context of WKY rats and treatment-resistant depression.

Additionally, the identification of strain-specific and interaction effects emphasizes the complexity of depression and the need for further research to unravel the underlying molecular mechanisms and develop targeted interventions for treatment-resistant depression.

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P.1-13. Antibody-drug conjugates as a viable strategy for off-target toxicity reduction in cancer treatment

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Background: In recent years, targeting the UPS (Ubiquitin-Proteasome System) has emerged as a rational approach in the treatment of cancer, as inhibition of protein degradation leads to cell death. CPL-410-005 compound was designed and developed as a non-selective and cell-permeable E1 ubiquitin ligase small-molecule inhibitor. Antibody-drug conjugates (ADCs) are a viable tool used for targeting toxic payloads directly to cancer cells, effectively improving selectivity, and limiting off-target toxicity in cancer therapy. Here, we present a CPL-410-005-antibody drug conjugate, with an improved toxicity profile to non-cancer cell lines, in comparison to unconjugated CPL-410-005.

Material and methods: A small molecule CPL-410-005 was designed in Celon Pharma as a novel inhibitor of E1 ubiquitin ligase, and CPL-410-077, a conjugation-ready derivative. Western blot analysis was used to assess the polyubiquitination levels in cells treated with our compound and MLN7243, as a reference. Compounds cytotoxicity was evaluated with CellTiter Glo on the SK-BR3 (Human Breast Cancer), SK-OV-3 (Human Ovarian Cancer), and HEK-293 (Human Embryonic Kidney), cell lines. CPL-410-077 and antibody ADC were characterized in vitro for the interaction with the target receptor using an SPR (Surface Plasmon Resonance). ADCs were then tested again in cell viability assay, to determine off-target toxicity levels in HEK293.

Results: CPL-410-005 at 250 nM inhibits cellular polyubiquitylation with greater potency than MLN7243. The cytotoxic activity of CPL-410-005 was displayed strongly in SK-BR3 (IC50=0,017 μM) and SK-OV-3 (IC50=0,04 μM). In HEK293 CPL410-005 cytotoxicity was observed (IC50=0,118 μM) and significantly reduced in a conjugate format CPL-410-077-antibody (IC50=4,4 μM). Conjugation did not affect the antibody's binding properties, with low-nM dissociation constants.

Conclusion: The biological potency and selectivity of the compound were evaluated in a cell viability test, western blot, and SPR analyses. The CPL-410-077-antibody conjugates may provide selective antitumor effects for targeted therapy, with greatly reduced toxicity. The presented strategy allows for high flexibility in targeting different cancer types in possible future therapies, with enhanced safety by reducing off-target side effects compared to free CPL-410-005.

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P.1-14. Dual inhibitors of anthracyclines reducing enzymes (AKR1C3,CBR1) with chemosensitizing, proapoptotic and cardioprotective activity

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Background: The search for effective and safe anticancer therapies is one of the biggest challenges of modern oncology. Anthracycline antibiotics (ANT), especially doxorubicin (DOX) and daunorubicin (DNR) are among the most widely used group of anticancer drugs. Unfortunately, ANT biotransformation (C13 carbonyl group reduction) catalyzed by carbonyl reductase 1 (CBR1) and aldo-keto reductase 1C3 (AKR1C3), leads to the formation of metabolites with significantly reduced anticancer activity and potential cardiotoxic properties. Inhibition of the metabolism can lead to the improvement of the efficacy and safety profile of ANT.

Material and methods: The aim of the study was to evaluate the cytotoxic and cardioprotective effects of co-administration of novel CBR1 and AKR1C3 inhibitors, together with daunorubicin (DNR) or doxorubicin (DOX). Tested compounds were selected based on in silico assessment, using enzymes' optimized 3D models and the results of prospective virtual screening; further, their inhibitory properties against CBR1 and AKR1C3 were confirmed using recombinant enzymes. Compounds were evaluated against A549 non-small cells lung cancer cell line and H9c2 rat cardiomyocytes cell line by MTT viability, caspases activity assays and Hoechst 33342 staining.

Results: As a result of in vitro evaluation, we detected that co-administration of compounds ASP9521 (IC50 against AKR1C3: 0,36 µM; IC50 against CBR1: 44,0 µM), P18 (IC50 against AKR1C3: 14,52 µM) and P25 (IC50 against AKR1C3: 13,83 µM) significantly decreased IC50 of DAUN in A549 cell line. Effect of tested compounds on the induction of apoptosis in cancer cells was observed. Additionally, compounds ASP9521, P18, P7 (IC50 against AKR1C3: 10,72 µM) and C18 (IC50 against AKR1C3: 6,27 µM; IC50 against CBR1: 62,80 µM) protected H9c2 rat cardiomyocytes cell line from the cardiotoxic effect of DOX and DAUN.

Conclusions: These initial in vitro evaluation proves that use of new chemotypes of anthracycline antibiotics reductases inhibitors have a potential to enhance effectivity and safety of anticancer therapy. Further studies on structure optimization and structure-activity relationship are necessary to obtain inhibitors exhibiting more potent pharmacological activity.

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P.1-15. Each answer has to be justified - a machine-learning based platform for evaluation of compound ADMET properties with explainability module

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Background: Great progress in the development of computational strategies for drug design application has revolutionized the process of the search for new drugs. Although the focus of in silico strategies is still put on the provision of the desired activity of a compound to the considered target, characterization of a compound in terms of its physicochemical and ADMET properties, becomes an indispensable element of computer-aided drug design protocols.

Materials and methods: A series of regression machine-learning-based predictive models was developed for the *in silico* assessment of selected ADMET features: cardiotoxicity, hepatotoxicity, solubility, biological membranes permeability, and metabolic stability. Their predictive power was tested in the cross-validation studies with the use of the data deposited in the ChEMBL database. In addition, the LIME and counterfactual analysis was performed and on its basis the influence of particular structural moieties on the evaluated properties and on the obtained predictions was examined.

Results: The efficiency of predictive models varied depending on the evaluated property and the machine learning algorithm used and the explainability module enabled identification of chemical moieties influencing the evaluated property to the highest extent. The developed tools were made publicly available in the form of an on-line platform for ADMET-based assessment of compounds.

Conclusions: An *in silico* platform was developed for the prediction of ADMET properties, being of great importance in the drug design process. The explainability module included can help in guiding the process of compound optimization and the tool can be widely used by medicinal chemists thanks to its availability on-line and intuitive graphical user interface.

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P.1-16. Amorfrutin B modulates PPARγ expression and protects neurons against hypoxia/ischemia by inhibition of autophagy as well as oxidative stress

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Background: Annually, 15 million people suffer from stroke worldwide of which 5 million die and 5 million are permanently disabled. Ischemic stroke is treated with rtPA that dissolves blood clot; however, narrow therapeutic window (4.5 h) and several contraindications prompt scientists to look for new therapy. Due to hypothesis linking stroke with metabolic impairment, we propose targeting metabolic-related PPARγ signaling through new SPPARγM, amorfrutin B. Through the high binding affinity to the receptor and safe pharmacological profile devoid of side effects typical of full PPARγ agonists, amorfrutin B could become an excellent tool to selectively modulate PPARγ signaling and to evoke neuroprotection in stroke.

Material and methods: In our study, we assessed the neuroprotective mechanism of action of amorfrutin B that was applied as post-treatment starting 6 h after hypoxia/ischemia. To determine the effect of amorfrutin B on oxidative stress and autophagy we determined level of 8-OHdG (ROS-related DNA damage marker) and the degree of formation of autophagolysosomes. The involvement of PPARγ, PGC1α, ADIPOQ and HIF1α in amorfrutin B-induced neuroprotection was assessed with the qPCR, ELISA and western blot analyses.

Results: Post-treatment with amorfrutin B decreased 8-OHdG level and autophagolysosomes formation indicating neuroprotective effect against hypoxia and ischemia. Amorfrutin B increased the protein level of PPARγ during hypoxia but decreased the mRNA and protein levels of PPARγ during ischemia. This substance also strongly inhibited *Hif1α*/HIF1α expression during hypoxia, while it did not affect this factor during ischemia. No correlation was found between PPARγ expression and the expression of PPARγ-regulated factors, i.e., PGC1α and ADIPOQ.

Conclusions: We showed for the first time the neuroprotective capacity of amorfrutin B against hypoxia and ischemia that relies on selective modulation of PPARγ and inhibition of oxidative stress and autophagy. This effect is accompanied by modulation of PPARγ and HIF1α signaling pathways particularly during hypoxia. Our study indicates that selective modulation of PPARγ *via* amorfrutin B may represent a novel and safe therapeutic approach based on widening therapeutic window as well as multifactorial action against stroke-induced neurodegeneration.

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P.1-17. The role of brain glutamatergic NMDA receptors in the regulation of cytochrome P450 in the liver

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Background: Cytochrome P450 (CYP) is a superfamily of enzyme hemoproteins metabolizing endogenous substrates and xenobiotics including drugs. Our previous studies carried out after intraperitoneal administration of the selective antagonist of GluN2B subunit of NMDA receptor CP-101,606 suggested that apart from monoaminergic systems, the brain glutamatergic system may also contribute to the regulation of liver cytochrome P450. In order to distinguish between central and peripheral mechanisms of enzyme regulation, in the present study we aimed at investigation whether intracerebral treatment with CP-101,606 can affect cytochrome P450 in the liver.

Methods: The experiment was carried out on male Wistar rats. Guide cannulas were implanted bilaterally into the lateral brain ventricles. The animals were given CP-101,606 (6, 15 or 30 µg/brain) for 5 days. The activity of cytochrome P450 was measured in liver microsomes, based on velocity of metabolic specific reactions. The CYP protein levels in liver microsomes were detected by Western blotting, serum hormone levels were measured by ELISA.

Results: CP-101,606 given into brain lateral ventricles exerted dose-dependent effects on the activity and protein level of liver cytochrome P450. CP-101,606 injected in the lowest dose increased the activity and protein level of CYP2C11 compared to the control and to the higher antagonist doses. The lowest dose of CP-101,606 also increased the activities (but not protein levels) of CYP2C6 and CYP2D compared to the control and to the higher antagonist doses. The activity of CYP2A was enhanced after the lowest dose compared to the highest dose. Moreover, the CYP1A and CYP2B protein levels (but not activities) were augmented after the lowest or the highest dose, respectively. CP-101,606 did not affect CYP3A. At the same time, a significant increase in growth hormone and a decrease in corticosterone concentrations were observed.

Conclusions: The presented results indicate new physiological mechanism of regulation of liver cytochrome P450 by the brain glutamatergic system *via* NMDA receptor, and suggest involvement of endocrine system in this regulation. The obtained results may be of practical application when predicting changes in cytochrome P450 activity and possible drug-drug interactions evoked by drugs affecting NMDA receptors.

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P.1-18. Design and synthesis of multitarget serotonin and non-NMDA receptors ligands as a possible strategy for the treatment of anxiety disorder

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Anxiety disorder is a broad term that covers several different conditions, all of which are characterized by excessive and persistent feelings of fear, anxiety, or tension. It is a complex medical condition, caused by a combination of various factors and can have a significant impact on a person's mental and physical health. People with anxiety disorders may experience a range of symptoms, including irritability, difficulty concentrating, and sleep disturbances. These symptoms can be persistent and debilitating, leading to significant stress and impairment of daily functioning. Current pharmacological treatments such as selective serotonin uptake inhibitors (SSRIs), serotonin-norepinephrine reuptake inhibitors (SNRIs), $GABA_A$ receptor agonists (benzodiazepines) are not effective for all patients. Additionally, these medications can cause various side effects, including nausea, dizziness, drowsiness, dry mouth, and sexual dysfunction. As a result, the search for new and improved anxiolytic drugs is ongoing, and alongside this, novel biological targets are being proposed.

Serotonin receptors 5-HT_7 and 5-HT_6 have been identified as attractive targets for novel anxiolytic drugs due to their involvement in the modulation of anxiety-related behaviors. Recently, other new interesting novel targets for the development of anxiolytic drug targets has also been proposed, e.g. the inhibition of ionotropic non-NMDA glutamate (AMPA/KA). In our previous research, we have developed the hydantoin derivative **PPK-29** – a highly potent ligand of the 5-HT_7 receptor (K_i = 7 nM). This study focuses on design and synthesis of novel analogues of compound **PPK-29,** with modifications of the imidazolidinedione group to quinoxaline-2,3-dione as a plausible bioisoster substitute. Quinoxaline-2,3-dione core is a known pharmacophore of AMPA/KA receptors described in various studies and such a modification could possibly lead to novel generation of ligands with multiple action at serotonin- and non-NMDA receptors. Desired analogues were obtained through a five-step synthesis pathway involving epoxide ring opening, nucleophilic aromatic substitution, reduction reaction and spontaneous ring closure. Further, compounds was submitted for *in vitro* testing to evaluate their binding affinity and efficacy at serotonin receptors as well as native ionotropic glutamate receptors.

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P.1-19. Analgesic effects of novel histamine H_3 receptor antagonist is correlated with changes in spinal dopamine level in a murine model of neuropathic pain

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Background: Neuropathic pain is still a challenge for the world of medicine. This chronic condition contributes to a decrease in people's live comfort and what is more disturbing it demonstrates resistance to available therapies. Recent, studies have indicated that histamine and its receptor have been connected to nociceptive transmission. The aim of our research was to evaluate the effect of the novel H3R antagonist (E-98) on pain behaviour and spinal changes of dopamine (DA) and its metabolites (DOPAC, HVA, 3-MT) levels in mice model of neuropathic pain.

Materials and methods: Albino Swiss mice were subjected to chronic constriction injury (CCI) to the sciatic nerve. 7 days after after surgery, animals were chronically injected with the vehicle or E-98 (10 mg/kg, i.p., twice daily for the next 7 days). The analgesic effects of E-98 were tested in the von Frey test (mechanical stimuli) and cold plate test (thermal stimuli). Behavioural tests were performed on days 3rd, 5th and 7th after the first E-98 injection. After last morning treatment the ipsilateral lumbar spinal cord was collected for Hight-Performance Liquid Chromatography (HPLC) analysis of DA and its metabolites.

Results: Our behavioural studies revealed a strong analgesic effect of novel antagonist in both performed tests. The HPLC analysis showed decreased level of DA concentrations in lumbar spinal cords after chronic E-98 treatment as compared to vehicle-treated mice. The level of DOPAC and HVA remained unchanged, however, CCI strongly induced 3-MT level. Further analysis has shown an up-regulated level of DA reuptake measured by the index [3-MT]/[DA] and total DA metabolism indicated by [HVA]/[DA] index after E-98 treatments.

Conclusions: Here, we demonstrated the analgesic effects of a novel H3R antagonist, E-98, in preclinical model of neuropathic pain. Based neurochemical data we hypothesized that the mechanism responsible for the analgesic effect of E-98 is partially related to its influence on spinal dopamine level, as well as its reuptake and metabolism.

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P.1-20. vitro analysis of cytotoxicity and neuroprotective potency of theranostic nanocarriers for drug delivery in CNS disorders

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Background: Nanotechnology is innovative and fast developing area of science and its growing application in medicine covers diagnosis, imaging and drug delivery. Nowadays, nanoparticles (NPs) have attracted much attention as promising drug carriers that could deliver therapeutics to their specific molecular targets. Among different kinds of nanocarriers, the multifunctional theranostics NPs that are suitable for both diagnosis and therapy should be highlighted. The aim of this study was to evaluate the potential cytotoxic effect of two different types of theranostic nanocarriers with encapsulated neuroprotective drugs.

Material and methods: For initial tests we have chosen AOT/$(PLL/PGA)_2$-g-PEG and PCL/$(PLL/PGA)_2$-g-PEG nanoparticles without gadolinium, with single or double layers labelled with gadolinium. Moreover, we selected cyclosporine A (CsA) and tacrolimus (FK506) as neuroprotectants due to their anti-apoptotic, immunosuppressive and anti-inflammatory properties. Undifferentiated and differentiated human neuroblastoma SH-SY5Y cell line was used to evaluate the potential cytotoxic effect of the theranostic nanocarriers loaded with selected drug. Cytotoxicity of empty NPs and NPs loaded with drugs was evaluated by the cellular viability quantification and cell death assessment using WST-1 and LDH tests, respectively. Moreover, we examined the neuroprotective potential of encapsulated CsA and FK506 against oxidative stress-induced cytotoxicity in the same cell culture.

Results: The obtained experimental data on *in vitro* cytotoxicity and cell viability showed that empty NPs were only toxic at the lowest 10-fold and 20-fold dilution. The results clearly indicate that the application of one or two layers labeled with gadolinium does not increase the cytotoxicity of NPs. Furthermore, the encapsulated CsA and FK506 moderately reduced H_2O_2-evoked cell damage in SH-SY5Y cells.

Conclusions: The results of the *in vitro* study indicate that NPs in higher dilutions are devoid of cytotoxicity. Moreover, polymeric-based nanoparticles seem to be promising carriers for drug delivery and diagnosis in central nervous system disorders.

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P.1-21. Changes in myelination induced by a maternal high-fat diet during pregnancy and lactation as a potential mechanism of depression in rat offspring

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Background: According to the developmental origins of health and disease (DOHaD), early-life environmental exposures including maternal diet can contribute to the risk and severity of later-life disease in offspring. In recent years, there is strong evidence that exposure to a maternal high-fat diet (HFD) provokes changes in the structure, function, and development of the central nervous system and may provoke neuropsychiatric disorders in offspring, including depression. Since brain development occurs prenatally and during lactation, maternal nutrition has been identified as a key factor for brain growth and maturation, however little is known about the effect of HFD during gestation and lactation on the myelination in the offspring's brain.

Materials and Methods: In the present study, we will investigate the effects of maternal HFD (60% energy from fat) during pregnancy and lactation on depressive-like phenotype (forced swimming test, sucrose preference test) and myelin-related changes (next-generation sequencing of mRNA, RT-qPCR, and ELISA) in adolescent and adult rat offspring.

Results: Maternal HFD during pregnancy and lactation resulted in increased immobility time during the forced swimming test in both adolescent and adult offspring. The latter change is likely related to the dysregulation of myelin-oligodendrocyte glycoprotein, myelin and lymphocyte protein, and kallikrein 6 (gene and protein levels) in the prefrontal cortex of adolescent and adult offspring followed maternal HFD during pregnancy and lactation.

Conclusions: In summary, maternal HFD-induced changes in myelin-related genes and proteins seem to contribute to depressive-like behavior in adolescent offspring, which persists even to adulthood.

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P.1-22. Investigation of MRI Contrasting Properties of Potential Theranostic Nanocarriers

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Background: Magnetic resonance imaging MRI is named as the most efficient diagnostic modality and it can be enhanced when combined with added specific contrast agents or theranostic drug nanocarriers. The aim of this study was to check relaxation properties of nanocarriers with nanoemulsion and polymeric cores formed with the ionic surfactant AOT (docusate sodium salt) or with AOT and PCL (polycaprolactone) with different contrast agent: gadolinium labeled poly-L-lysine (PLL-Gd), iron oxide nanoparticles (Fe_3O_4), and third type of nanocarriers hybrid nanosilica HNS labeled with Gd atoms (Gd-HNS) to potential use as a theranostic nanocarriers.

Material and methods: The nanocapsules AOT/PCL and AOT were prepared via a layer-by-layer technique. Gadolinium labeled poly-L-lysine (PLL-Gd), iron oxide Fe_3O_4 were added as a MRI contrast agents to shell. On the other hand Gd-HNS nanoparticle were prepared by a two-step synthesis: controlled sol-gel synthesis of HNS nanoparticles followed by chemical conjugation of Gd. The imaging and relaxometry measurements were performed using *9.4T Bruker Biospec 94/20 MRI* scanner equipped with a birdcage RF coil.

Results: For nanocarriers containing gadolinium, the linear regression results for r_1 specific relaxivities were as follows: 8.11 ± 0.51 $mM^{-1}s^{-1}$ for PCL/AOT/PLL-Gd and 9.97 ± 0.13 $mM^{-1}s^{-1}$ for AOT/PLL-Gd. For Fe_3O_4-doped nanocarriers value of relaxivity is r_2 = 788 ± 25 $mM^{-1}s^{-1}$. For Gd-HNS nanocarriers both r_1 and r_2 relaxivity values (2.118 ± 0.026 and 105.0 ± 5.2 $mM^{-1}s^{-1}$, respectively) were close to those of commercially available contrast agents like Magnevist™ (r_1 = 3.3 - 3.7 $mM^{-1}s^{-1}$ at 3.0 T).

Conclusions: Incorporation of the Gd complexes, iron oxide nanoparticles compounds into shell of two types of polyelectrolite nanocapsules was confirmed as an effective means for enhancement of contrast in MRI- based imaging. Hybrid nanosilica labeled gadolinium Gd-HNS affects mainly the transversal relaxation. Our studies showed that polyelectrolyte nanocapsules with Gd complexes or iron oxide and Gd-HNS show great potential for theranostic application.

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P.1-23. Neuroprotective and immunomodulatory effects of human Mesenchymal Stem Cells (hMSC) secretome on epileptic mouse brain tissue

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Background: Treatment of drug-resistant epilepsy (DRE) in children still remains a considerable medical challenge. Classic pharmacotherapy has limited clinical efficacy and most notably it often impairs cognitive development of a child and therefore novel therapeutic strategies are being intensively sought. Human Mesenchymal Stem Cell (hMSCs)-based therapies have attracted much attention as a treatment of central nervous system (CNS) injuries and were proven to reduce the number and intensity of epileptic seizures in patients. Although the exact mechanism of hMSCs have not been fully elucidated, it is suggested that their therapeutic effect may be associated with pro-neurogenic and anti-inflammatory properties of hMSC secretome.

Material and methods: We investigated how hMSC secretome would influence the viability and inflammatory status of brain cells in Organotypic Hippocampal Cultures (OHC) isolated from NOD SCID mice with pilocarpine (PILO)-induced temporal lobe epilepsy (TLE). OHC were cultured in hMSC-conditioned OHC medium (hMSC-CM). Influence of hMSC-CM on OHC was performed by measuring selected markers associated with cell viability, neurogenesis and inflammatory response by the combination of biochemical, molecular and bioimaging techniques.

Results: Bioimaging of hippocampal slices using CLARITY technique revealed a decreased number of parvalbumin-positive neurons in OHC obtained from PILO-treated mice, thus suggesting GABAergic neuronal cell death the development of epileptogenesis. OHC cultured in hMSC-CM displayed an increase in the levels of nestin and downregulation of NFκB in both control and PILO-treated mice, when compared to control groups, cultured in standard medium It also prevented necrotic cell death, since it significantly decreased the levels of lactate dehydrogenase in the culture medium.

Conclusions: Obtained results indicate that hMSCs have beneficial effect on TLE-damaged mouse brain by improvement of cell viability, stimulation of neurogenesis and alleviation immune status of murine OHC slices. These findings strongly support hMSC-based therapy as valid therapeutic option for the treatment of TLE and outlines the role of hMSC secretome as key factor responsible for their beneficial effect in the neuroregeneration.

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P.1-24. Histological analysis of selected organs of mice exposed to chronic immobilization stress and a zinc-restricted diet

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Background: Previous research has shown that restricting zinc in the diet just like chronic stress induces depressive-like behavior in rodents. On the other hand, available data from the literature suggest that one of the mechanisms underlying this effect may be the induction of inflammatory processes. The aim of the study was to determine whether stress and zinc deficiency are involved in histological changes in the intestines and immune organs: thymus and spleen.

Material and methods: Mice (C57Bl/6) were randomly assigned to four treatments: ZnA-control group receiving pellets containing 50mg/kg of zinc, ZnA/CRS - animals exposed to 3 weeks of chronic restrain stress (3h/d) and the same diet as the previous group, ZnD/CRS- exposed to the same stress procedure and the diet containing < 3mg/kg Zn for 4 weeks and ZnD- receiving a diet with zinc level < 3mg/kg. After the end of the experiment,the weight of the animals, spleen, and thymus was measured. Both organs and the intestines were stained with hematoxylin-eosin for morphometrical analysis. An alcian blue was used to visualize and count the goblet cells in the intestinal sections

Results: Both stress and zinc restriction and a combination of both led to significant body weight loss (ZnA-27.05g *vs.*ZnA/CRS-24.01, ZnD/CRS-22.95, ZnD-22.93g).We observed a decrease in thymus weight in all stressed groups in comparison to non-stress animals (ZnA-0.0368, ZnD-0.0352 *vs*. ZnA/CRS-0.0254, ZnD/CRS-0.0225g). Zinc restriction led to the reduction in the number of thymocytes when compared to the ZnA/CRS group within both layers: cortex (ZnD-161.16 *vs.*ZnA/CRS 205.17 no. of cells/1000 μm^2) and medulla (ZnD-79.56 *vs*. 104.207no. of cells/1000 μm^2). Stress together with zinc restriction caused a decrease in spleen weight compared to the control group (0.0714 *vs*. 0.0561g).In all groups, the number of white pulp (ZnA-58.34 *vs*. ZnD-59.08, ZnA/CRS-63.47, ZnD/CRS-69.06 no. of cells/1000 μm^2) and red pulplymphocytes (ZnA-37.92 vs. ZnD-42.85, ZnA/CRS-42.86, ZnD/CRS-48.203 no. of cells/1000 μm^2) of the spleen increases.Zinc restriction led to a decrease in the number of Goblet cells in the large intestine (ZnA-6.41 vs. 4.72 no. of cells/50 µm).

Conclusions: Based on the results, it can be concluded that both chronic stress and zinc restriction in the diet causes morphological changes in the intestines and immune organs, which may affect the properties of the cells of the immune system and gastrointestinal tract.

P.1-25. Maternal high-fructose diet prone male rats to cocaine addiction

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Background: Cocaine use disorder (CUD) is the compulsive use of cocaine that cause major medical, psychological, and behavioural problems. CUD affect people across genders and ages, imposing significant barriers to a person's ability to function normally. Individual mental health predisposition is one of the crucial predictors of CUD development. In a recent review, diet patterns, especially maternal high-sugar consumption, predispose to the development of mental illness and prone to substance use disorders (SUD), such as CUD. Hence, our preclinical research aimed to examine the impact of maternal fructose (FRU; 54%) diet consumption on SUD behavioural predictors and cocaine-seeking behaviour in offspring.

Material and methods: Wistar male offspring rats after maternal FRU diet during pregnancy and lactation were evaluated either in the behavioural screening tests or in the intravenous cocaine self-administration (COC SA) procedure. Parallelly, locomotor activity, novel object recognition, elevated zero maze test and forced swimming test were conducted. The reinstatement of cocaine-seeking behaviour was assessed after COC SA with a stable dose of cocaine and an increased schedule of reinforcement procedures and extinction training.

Results: Our findings indicated that the maternal FRU diet led to hyperactivity, novelty-seeking, anxiety-like, and depressive-like behaviour alternation in male offspring. During COC SA maternal FRU diet evoked enhancement to reinforcement sensitivity to cocaine properties. Furthermore, FRU male offspring increased active lever presses after cue- and cocaine-induced reinstatement.

Conclusions: Our results showed that perinatal offspring exposure to the maternal FRU diet can change the emotional status of offspring. Herein, perinatal FRU intake is prone to change in the sensitivity to addictive drugs, and enhanced cocaine-seeking behaviour. We emphasize the inappropriate role of FRU in the maternal diet on the offspring's brain function development.

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P.1-26. The influence of resveratrol on kynurenine pathway in rat brain- HPLC and gene expression chemometric study

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Background: Resveratrol (3,4',5-trihydroxy-trans-stilbene, RSV) is classified as a natural polifenolic coumpund produced by plants from Vaccinium spp. (redberries), Vitis spp, and families Cyperaceae or Gnetaceae. Its presence in red wine is considered as a basis of the French paradox. Besides antioxidant properties RSV displays potential pharmacology effects including anticancer, neuroprotective and anti-inflammatory. The main route of tryptophan (TRP) metabolism is towards kynurenine (KYN) and kynurenic acid (KYNA). KYN, a precursor of KYNA and an agonist of aryl hydrocarbon receptor (AhR) is associated with inflammatory, neurodegeneration or carcinogenesis whereas KYNA was shown to display potent neuroprotective and anticonvulsive properties and its impaired production was implicated in the pathogenesis of epilepsy and neurodegenerative disorders. It is suggested its anti-inflammatory and antioxidant properties. Enzymes involved in kynurenine pathway include kynurenine aminotransferases (KATs) and kynurenine 3-monooxygenase (KMO).

Material and methods: The research was conducted on male Wistar rats. After 14 days of daily intraperitoneal administration of RSV the rats were decapitated. Brain cortex, hippocampus and striatum was separated and the amount of KYNA, KYN and TRP was measured by HPLC with fluorimetric detection. RNA extraction from brain for investigation of enzymes of kynurenine pathway was performed using spin-column kits. Synthesis of single-stranded cDNA from tRNA was conducted on high-capacity cDNA reverse transcription kits. Amplification and quantification of KAT I, KAT II, KMO genes was carried out using a rt-PCR system.

Results: The analysis of interaction between RSV, content of TRP, KYNA, KYN in brain and KAT I, KAT II and KMO genes expression was conducted using principal component analysis (PCA). Projection of the variables onto the plane shows positive correlation between KATs genes expression and the amount of KYNA in the corresponding organ, for brain cortex and hippocampus, but not for striatum. TRP concentration in brain cortex, hippocampus and striatum was also intercorrelated positively, furthermore the PCA shows the separation of lower doses of RSV from control group and higher doses.

Conclusions: Assumed correlations between TRP, KYNA, KYN levels and KAT I, KAT II and KMO genes expression caused by RSV administration were observed.

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P.1-27. Effect of psilocybin and ketamine on rat limbic neurotransmission and behaviour

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Background: Mood and anxiety disorders are one of the most common threats to mental health, while expressing high levels of comorbidity. Both ketamine and psilocybin seem to exhibit rapid antidepressant and anxiolytic effect, in comparison to currently used drugs. Either ketamine and psilocybin affect connectivity between the prefrontal cortex and limbic system involved in depression.The aim of this study was to assess the effect of psilocybin on behavior and neurotransmission in hippocampus, nucleus accumbens and amygdala, in comparison to ketamine.

Materials and methods: Microdialysis was performed in freely moving rats. Probes were implanted into the hippocampus, nucleus accumbens and amygdala. Seven days later, probes were connected to a syringe pump delivering artificial cerebrospinal fluid. After establishing baseline animals were injected with ketamine (10 mg/kg, ip.) or psilocybin (2 or 10 mg/kg, sc.) and fraction collection continued for 240 minutes. Dialysates were analyzed using HPLC with electrochemical detection. The open field test was conducted on 1 hour after the injection of drugs and animal behavior was recorded for 5 min.

Results: All substances elevated extracellular levels of dopamine, serotonin and GABA in the nucleus accumbens and hippocampus. Glutamate level was decreased by psilocybin in the nucleus accumbens, by the lower dose in the hippocampus and during the 1sth after administration in amygdala. Ketamine increased glutamate and GABA in the nucleus accumbens, hippocampus and in the 1sth after administration in amygdala. Psilocybin and ketamine significantly increased extracellular level of acetylcholine in the hippocampus.

Psilocybin dose dependently decreased the locomotor activity and exploration of rats 1 h after administration while increased the time spent in the center as a measure of anxiety. Ketamine affected only locomotor activity increasing it. Increase in center exploration was observed by all substances 24 h after administration.

Conclusions: Both drugs significantly changed neurotransmission in the limbic regions of rat brain. Increase in dopamine and serotonin extracellular levels in the nucleus accumbens as well as predominant GABAergic neurotransmission in limbic regions accounts for antidepressant and anxiolytic effects of psilocybin. Elevation of hippocampal acetylcholine suggests beneficial effects on rats cognitive functions.

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P.1-28. Novel fenoterol analogs as potent inhibitors of MAPK signaling in melanoma cells

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Background: The β2-adrenergic receptor (β_2AR) signaling has been implicated in the regulation of critical processes in the development and progression of cancer, such as cell proliferation, apoptosis, and angiogenesis. Fenoterol constitutes a potent scaffold for probing the activity of the β_2AR. Previously, we have generated a series of fenoterol analogues and stereoisomers to generate new highly selective agonists of the receptor. Here, aimed to assess the capacity of a panel of fenoterol analogues to inhibit proproliferatory ERK signaling in cultured melanoma cell in order to identify correlation between the structure and the inhibitory activity towards the mitogen-activated protein kinase (MAPK) signaling pathway. By gaining a better understanding of the mechanism of action of the fenoterol derivatives, this study may contribute to the ongoing efforts to develop more effective therapies for cancer.

Material and methods: Human UACC-647 melanoma cells were treated for 20 min. with an increasing concentrations of 17 fenoterol derivates, forskolin, isoprenaline, or vehicle (DMSO, 0.1%). Phosphorylation of ERK signaling node was studied by western blotting.

Results: Structure–activity relationship of ligand-induced dephosphorylation towards of ERK indicates that (*R*,*R*') stereochemistry of molecules the most important for promoting the tested activity. Derivatives bearing naphthyl ring show the highest activity expressed as IC_{50} values. (*R*,*R*')-4'-hydroxy-1-naphthylfenoterol yielded IC_{50} = 15.7 pM, whereas (*R*,*R*')-1-naphthylfenoterol and (*R*,*R*')-4'-methoxy-1-naphthylfenoterol generated the IC_{50} values of 39.5pM and 61.7pM, respectively. Swapping methyl moiety into ethyl or propyl group significantly reduces the inhibitory action of the compounds toward ERK.

Conclusions: Our study provides valuable insights into the potential of fenoterol derivatives ERK modulators and identifies β_2AR as a potential target for anticancer drug development. Overall, our findings suggest that (*R*,*R*')-4'-hydroxy-1-naphthylfenoterol and some other fenoterol analogs are promising candidates for β_2AR-dependent suppression of ERK signaling.

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P.1-29. The olfactory bulb drives NMDA receptor antagonist fast oscillations in the piriform cortex in freely moving rats

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Background: NMDAR antagonists, such as ketamine and MK801, enhance high-frequency oscillations (HFO, 130-180 Hz) in many brain regions of freely moving rats. Our recent studies show that the olfactory bulb (OB) is an important generator of this rhythm. The aim of the study was to examine whether systemic injection of MK801 could enhance HFO in the piriform cortex (PC), one of the main targets of the OB, and to what extent the OB drives activity in this area.

Material and methods: Simultaneous local field potentials were recorded from the OB and PC before and after systemic injection of 0.15 mg/kg MK801 in freely moving male Wistar rats. Thirty minutes after MK801 injection rats received microinfusion (0.5 microg/saline) to the OB or PC. Tetrodotoxin was also infused to rats with guides in the PC.

Results: Systemic injection of MK801 increased the power of HFO in both the OB and the PC which was significantly higher in the OB compared to the PC. Microinfusion of muscimol to the OB produced an immediate reduction in HFO power in both the OB and PC. Changes in HFO power in the OB and PC positively correlated. Further, slow rhythms in the OB drove HFO locally and in the PC. Muscimol infusion to the PC produced negligible changes in HFO power in the PC or the OB. By contrast, TTX produced an immediate reduction in HFO power selectively affecting the PC.

Conclusions: The activity of OB efferent pathways can drive HFO, after NMDAR antagonist injection, in a major downstream target. These findings are in line with our other studies demonstrating the OB plays a crucial role in broadcasting this activity to other brain areas.

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P.1-30. The Role of Epigenetic Readers, Bromodomain and Extra-Terminal Motif Proteins, in the Development of Schizophrenia

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Background: Structural and functional abnormalities observed in schizophrenia are considered the result of early-life developmental disruption, and the dysfunction of epigenetic mechanisms such as DNA methylation and histone modifications appear to be involved in the pathomechanism of this mental disorder. Epigenetic readers of acetylation marks from the bromodomain and extra-terminal domain (BET) family, comprised of four proteins: BRD2, BRD3, BRD4, and BRDT, are key regulators of chromatin dynamics and the disease-associated acetylome. Accumulating evidence suggests that treatment aimed at alleviating BET protein interactions with hyperacetylated histones may aid in the prevention or treatment of schizophrenia.

Material and methods: The present study investigated the role of BET proteins in schizophrenia-like abnormalities in a neurodevelopmental model of schizophrenia induced by prenatal methylazoxymethanol (MAM) administration (MAM-E17). An inhibitor of BET proteins, JQ1, was administered during adolescence on postnatal days (P) 23–P29, and behavioural responses (sensorimotor gating, recognition memory), as well as molecular and structural alterations in the medial prefrontal cortex (mPFC) (transcriptomic and proteomic analyses accompanied with microscopic imaging), were studied in adult males and females.

Results: Imaging studies of the BET protein expression revealed that BRD4 is expressed throughout the brain and present in neurons and generally not seen in glial cells. Deficits in sensorimotor gating and recognition memory were observed mainly in males in all analysed groups, however, the strongest impairment in the recognition memory was detected in the VEH-JQ1 groups of both sexes. A proteomic study as well as a transcriptomic analysis employing the RNA-seq technique disclosed the effect of MAM on neuroplasticity-related genes and the suppression of immediate early gene expression by JQ1.

Conclusions: MAM-induced schizophrenia-like abnormalities were observed only in males, while adolescent JQ1 treatment altered behavioural responses and the molecular and proteomic landscape in the mPFC of both sexes. Thus, transient adolescent inhibition of the BET family might prompt permanent alterations in the mPFC and develop schizophrenia-like malfunctions.

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Preclinical Research – Other themes

P.1-31. Pharmacological treatment with autophagy inhibitor bafilomycin A1 reduced cardiac hypertrophy in mouse model of experimental autoimmune myocarditis

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Background: Dilated cardiomyopathy is a progressive heart disease characterized by intensified fibrosis, which results in enlargement and stiffening of the chambers and deterioration of contractile function. Autophagy is a catabolic process resulting in the degradation of damaged cellular components under homeostatic and stress conditions. So far, the role of autophagy in dilated cardiomyopathy progression is still undetermined, and the available literature data are inconclusive. The goal of the study is to evaluate the effect of the pharmacological autophagy inhibitor bafilomycin A1 (BafA1) on the development of myocarditis and its progression to dilated cardiomyopathy in a mouse model of experimental autoimmune myocarditis (EAM).

Material and methods: To induce EAM, 6-8 weeks old BALB/c mice were immunized with myosin heavy chain alpha together with complete Freund's adjuvant. BafA1 (dose of 2.5 µg/injection/mice) was applied in two approaches: preventive (administration from days 0 to 21, 3 times a week, analysis d21) and therapeutic (from days 17 to 40, 3 times a week, analysis d40). Inflammation and fibrosis severity were assessed on heart tissue sections with histological and immunohistochemistry staining. Furthermore, for ex vivo and in vitro experiments, Western-blot, real-time RT-PCR, ELISA, and proliferation assay techniques were used.

Results: In the preventive approach, histological staining revealed no significant effect of BafA1 on the development of myocarditis. However, a reduction in fibrosis and cardiomyocyte size was observed in the hearts of BafA1-treated mice in the therapeutic approach. The Western blot analysis presented lower levels of proteins related to autophagy (Lc3II/I) and inflammation (Il6) in both models. Additionally, in the therapeutic approach, treatments with BafA1 caused a decrease in profibrotic collagen type 1 and fibronectin production. In vitro studies with bone marrow-derived macrophages demonstrated that BafA1 had no effect on the production of proinflammatory cytokines. Furthermore, BafA1 treatment reduced the proliferation of T cells.

Conclusions: The obtained results indicate a protective effect of autophagy inhibition on myocardial fibrosis in EAM. The potential cardioprotective effect of BafA1 may be a starting point for pharmacological treatments targeting autophagy.

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P.1-32. The role of the endothelial glycocalyx in vascular dysfunction - studies using fluorescence microscopy

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Background: The endothelial glycocalyx (GLX) is a net of carbohydrates and proteins anchored to the endothelium. This structure fulfills multiple functions, such as controlling vascular tone or participating in mechanotransduction. However, the most significant role of the GLX is acting as a protection of the endothelium due to its unique position in the vessels.

It has been confirmed that new antidiabetic drugs called sodium-glucose cotransporter 2 inhibitors (SGLT2-Is), one of the sodium management regulators, positively affect the endothelium, however, their influence on the GLX integrity remains unknown. Therefore our research assumes the usage of the SGLT2-Is and another sodium level regulator, cariporide – sodium-hydrogen exchanger inhibitor (NHE-I) – to investigate whether SGLT2-Is have a pleiotropic effect on the GLX, and whether lowering sodium levels suppress the GLX disruption during TNF-triggered endothelial inflammation.

Materials and methods: Here we are developing a complex methodology for the GLX visualization in an isolated murine blood vessel in physiological and pathological conditions using fluorescence microscopy. This method provides comprehensive information about the GLX and its alterations, including the impact of SGLT2-Is on the GLX integrity maintenance and changes in the cell sodium management.

Results: The heparan sulfate (HS, one of the main GLX components) coverage significantly decreases during TNF-triggered endothelial inflammation in comparison to untreated aortae samples after 1 and 4 h of incubation. The usage of empagliflozin (Empa, one of the SGLT2-I; 10 µM) and cariporide (Cari; 10 µM) in TNF-induced inflammation partially rebuilt the HS coverage, but only after 4 h of incubation. More interestingly, the level of HS coverage recovered by Empa and Cari remains the same, which raises the question of whether SGLT2-I acts *via* the NHE, not *via* the SGLT2.

Conclusion: In summary, sodium management in cells influences the GLX. This work adapted a microscopy-based methodology to study GLX with the aim of characterizing the effect of the SGLT2-I drugs and lowering sodium levels on GLX during endothelial inflammation.

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P.1-33. High-resolution spatial profiling of μ-opioid receptor transcripts in the murine forebrain

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Background: The μ-opioid receptor gene, *Oprm1,* has multiple transcriptional variants, with reported alternative transcription starts, ends and over 20 potential exons. While most of these variants include region encoding the seven transmembrane helices, there is a significant variability in the proposed C-terminus encoding sequences and the 3'untranslated mRNA regions. The existence of multiple isoforms raises the possibility that they could be differentially targeted with opioids, thus both offer an explanation for the observed differences in action as well as an opportunity for the development of novel drugs with reduced adverse effects. Here, we comprehensively analyzed the transcripts of the μ-opioid receptor (*Oprm1*) in the murine forebrain.

Methods: RNAseq transcription analyses were performed using Oxford Nanopore Sequencing (ONS) and Visium 10x Genomics. Spatial distribution of the selected exons was evaluated with RNAscope *in situ* hybridization. Allen's V1 & ALM - SMART-SEQ data set was used to conduct cell-specificity analysis.

Results: In Visium 10x Genomics sequencing, the alignment of reads to the reference genome showed a mismatch between annotated and observed mRNA ends. The major potential 3'terminus was observed at chr10:6,860,027 (GRCm38/mm10), which is ~9.5 kilobases downstream of the annotated exon 4 end. ONS revealed that the final *Oprm1* exon includes a 10.2 kilobase long 3'untranslated region consistent with the Visium 10x Genomics results. The presence of the long variant was confirmed using RNAscope *in situ* hybridization. The novel 3'UTR was observed in all brain regions examined, i.e. the thalamus, striatum, and cortex. Additional variants of the *Oprm1* gene were also observed, but their expression was close to the detection limit. Reanalysis of single-cell sequencing data from the Allen database validated our observations, and showed that *Oprm1* was expressed mainly in parvalbumin-, somatostatin- and VIP-positive cells.

Conclusion: We show that the primary *Oprm1* transcript in the mouse forebrain has a previously unannotated long 3'UTR. Expression of other variants appears minimal.

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P.1-34. The impact of new chlorinated derivatives of 2'-OH chalcone on model lipid membranes

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Chalcones are intermediate products in the biosynthesis of flavonoids, which exhibit a wide range of biological activities, including anti-inflammatory and antibacterial activity. During an inflammatory process in the organism flavonoids can react with the hypochlorous acid produced by neutrophils, forming stable mono- or di-chlorinated derivatives. In addition, it is considered that chlorinated derivatives of flavonoids may exhibit greater biological activity than their non-chlorinated counterparts. These reports prompted us to undertake research on the biological activity of new chlorinated derivatives of 2'-OH chalcone in relation to model lipid membranes. The activity will be determined based on the effects of their interaction with the membrane.

Four chlorinated derivatives of 2'-hydroxy chalcone were selected for this study (2'-hydroxy-5'-chloro chalcone, 2-chloro-2'-hydroxy chalcone, 3-chloro-2'-hydroxy chalcone, and 4-chloro-2'-hydroxy chalcone). The effects of these compounds on the POPC membrane were determined based on their ability to modify membrane fluidity and dipole potential using the fluorescence method with DPH and RH421 probes. In addition, the ability of the compounds to aggregate lipid vesicles with different surface charges was determined using dynamic light scattering. Based on the obtained results, the relationship between chalcones' biological activity and the position of the chlorine atom substitution was determined.

The experiments showed that the used compounds caused a decrease in lipid membrane fluidity and its dipole potential. These changes depend both on the concentration of the compounds used and on the position of the chlorine atom substitution in the chalcone molecule. In addition, these compounds practically do not induce aggregation of lipid vesicles with different surface charges, suggesting that electrostatic interactions do not play a fundamental role in their interaction with lipid membranes.

The changes induced by the used compounds in membrane fluidity and dipole potential will result in changes in membrane permeability and the activity of membrane proteins. Therefore, the tested compounds can be evaluated as substances with high biological activity and potential therapeutic effects.

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P.1-35. Mu-opioid receptor-dependent changes in social reward across adolescence in mice

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Background: In humans, adolescence is a time of rapid behavioral and neural changes, including a transient decrease in affect associated with being among family members. Here, we aimed to answer two questions. First, we asked if similar changes occur in mice. Specifically, we described changes in the rewarding effects of interactions with siblings in adolescent male and female mice. Second, we asked if these changes depend on the activation of the mu-opioid receptors.

Materials and Methods: Male and female mice of the C57BL/6 strain were used. Four developmental time points were studied: pre- (around post-natal day 33 [P33]), early (P36), middle (P39) and late (P42) adolescence. Rewarding effects of interactions with siblings were tested using the social conditioned place preference test (sCPP). The role of mu-opioid receptors was studied using a selective mu-opioid receptor antagonist, cyprodime (1 mg/kg), applied 1 hour before the post-test of the sCPP test.

Results: The rewarding effects of interactions with siblings in adolescent male mice followed a similar course as in humans: high in pre-adolescence, decreased in early- and mid-adolescence and returned to the initial level in late adolescence. In female mice, the lowest level of social reward was observed in late adolescence. Treatment with the opioid antagonist cyprodime increased the expression of social conditioned place preference in early adolescent mice, but not in older animals.

Conclusions: Taken together, we show similarities between mice and humans in the developmental changes of sensitivity to the rewarding effects of interactions with familiar kin, and demonstrate the involvement of the endogenous opioid system in the regulation of adolescent social behavior.

P.1-36. The role of the peptide LL-37 in modulating the immune response to extracellular nucleic acids present in the Candida albicans biofilm

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Background: Candida albicans is the most common cause of fungal infections worldwide. The high invasiveness and resistance to drugs are associated with biofilm formation. The main feature of the biofilm is the production of the extracellular matrix (ECM) made of polymers and extracellular nucleic acids (eDNA, eRNA). ECM components guarantee adhesion to surfaces and acts as impenetrable barrier. The development of biofilm drives the migration of neutrophils and macrophages and may activate various fighting mechanisms like phagocytosis, cytokine production, netosis (specific for neutrophils) and LL-37 biocidal peptide releases. The positively charged peptide LL-37 is known for its properties of forming a complex with nucleic acids, which can affect the cell response to these molecules.

Material and methods: Fungal nucleic acids were isolated using TRI-Reagent or purified by chromatography. Neutrophils were isolated using LSM medium and incubated with stimulants for 3 h. The viability was determined using the CellEvent™. Netosis was analyzed using Sytox Green dye, and ROS production using dihydrorhodamine. Cytokine production was analyzed using ELISA. THP-1 macrophage-like cells were cultured in RPMI (10% FBS + PMA) for 48 h and stimulated for 24 h before RT-PCR.

Results: The results indicated that neutrophil stimulation with eDNA/eRNA:LL-37, on the one hand, leads to attenuation of some types of responses (netosis, ROS) compared to "naked" molecules. On the other hand, the presence of the LL-37 peptide drives the phagocytosis of nucleic acids and increases the production of IL-8. In the case of THP-1 cells, LL-37 significantly enhance the interferon production in response to nucleic acids.

Conclusions: Despite the limited penetration of the biofilm by immune cells, the LL-37 peptide acts as a factor that complexes the yeast nucleic acids present in the biofilm, leading to better recognition of yeast infection and activation of an enhanced pro-inflammatory response.

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P.1-37. A systematic study on the ring conformational distortion in L-iduronic and L-guluronic acids and their derivatives

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Background: Uronic acids are building blocks in various biologically-relevant polysaccharides present in both plant and animal tissues. Identification of their dynamic molecular structure in aqueous solutions is essential for understanding their biological properties. Various methods for studying this issue are described in the literature, but they often lead to contradictory results.

Material and methods: We have considered six different compounds: 4 derivatives of L-iduronic acid (IdoA, IdoA-OMe, IdoA2S, IdoA2S-OMe) and 2 derivatives of D-guluronic acid (GulA2S and GulA2S-OMe). Calculations were carried out according to: (1) explicit solvent molecular dynamics simulation in a hybrid (QM/MM) potential; (2) two-step approach combining classical MD simulations (used as an engine to generate the conformationally-diverse set of configurations) and the subsequent QM calculations on representative structures, subsampled from classical MD trajectory. The two different schemes of weighting were applied with respect to the final average data obtained in approach. Calculations covered both the average conformational energies and the NMR parameters (*J*-coupling constant between vicinal pairs of protons).

Results: The most elastic rings belong to IdoA, IdoA-OMe, and GulA2S, where either $^{1}C_{4}$ conformer is preferred shape or there exists a dynamic equilibrium between all geometries, including $^{1}C_{4}$, $^{4}C_{1}$ and boat/skew-boat shapes. The quantitative energies of distortion vary strongly, depending on the method. The O-methylation usually shifts the equilibrium toward $^{1}C_{4}$, whereas sulfation acts in an opposite direction. The partial, average *J*-coupling constants (corresponding to particular ring shapes) were used to determine the exact content of each of the ring shape. Having the full relationship between torsional angles within pyranose rings and calculated *J*-coupling constants, we made attempts to refine the Karplus equations applicable to uronic acid systems.

Conclusions: We provide a series of quantitative data describing the conformational distortion in rings of uronic acids and demonstrated that accounting for both entropic- and solvent-related effects is essential for accurate determination the conformational equilibria in saccharides. Our approach can also be used for other small-molecule systems as well for potential refinements of the Karplus equations.

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P.1-38. Electrophysiological properties of dystrophin-deficient human induced pluripotent cell-derived atrial cardiomyocytes

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Background: Duchenne muscular dystrophy (DMD) is an X-linked genetic disorder caused by different mutations in *DMD* gene leading to absence of dystrophin. Patients with DMD demonstrate gradual damage of skeletal muscles and concomitant development of dilated cardiomyopathy, currently considered as a major cause of death in this disease. While predominant cardiac symptoms relate to the heart ventricles, around 30% of DMD patients are diagnosed with alterations in atrial electrophysiology including flutter and fibrillation. The thorough comparison of control and dystrophin-deficient human atrial cardiomyocytes, however, have not been described, therefore, the aim of our study was to fill this gap using human induced pluripotent stem cells (hiPSC).

Material and methods: Our *in vitro* model was based on hiPSC differentiated into cardiomyocytes by small chemical compounds inhibiting activity of GSK3b and WNT pathway, combined with retinoic acid-based specification into atrial phenotype (hiPSC-ACM). Evaluation of cell electrophysiology was performed on 3 pairs of isogenic control and dystrophin-deficient hiPSC-ACM lines, two of them with introduced deletion of exone 50 *DMD* and third one derived from DMD patient with deletion of exons 48-50 *DMD*. The effectivity of cardiac differentiation was performed by qRT-PCR and evaluation of *NR2F2, KCNJ3, KCNA5* as well as MYL7 immunochemical staining. Investigation of electrophysiology were performed by patch-clamp and Multielectrode Arrays (MEA).

Results: Performed analysis proved that addition of retinoic acid enhance differentiation into atrial cardiomyocytes. Control and DMD hiPSC-ACM demonstrated similar expression of major atrial markers including *NR2F2, KCNJ3, KCNA5* and were positive for MYL7. Additionally, patch-clamp analysis did not show changes in parameters like velocity of depolarization and repolarization at 20%, 50% and 90% of action potential, and no differences were observed in field potential duration, RR intervals and R-S slope measured by MEA. Despite lack of electrophysiological alterations between control and dystrophin-deficient hiPSC-ACM, we have observed increased expression of *KCNJ3* and decreased *NPPA* in the latter group.

Conclusions: In summary, DMD hiPSC-ACM did not demonstrate significant electrophysiological changes in comparison to their control counterparts, however, decreased expression of KCNJ3 and NPPA may indicate development of arrhythmia.

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P1-39. The molecular mechanisms of Alzheimer's disease neuropathology may be accelerated or induced by Western diet-derived metabolic disorders

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Background: Bad diet in known as a risk factor for Alzheimer's disease (AD), but the mechanisms remain not fully elucidated. The aim of the present study was to investigate and compare the effect of western diet (WD) on insulin signaling in the brain and on the development of AD in familial AD (FAD), i.e. in the presence of FAD mutation and in an unaltered genetic background during ageing (model of sporadic AD, SAD).

Material and methods: To this aim, the WD effects on the formation of pathological amyloid-β (Aβ) and tau protein phosphorylation in the brain were investigates in the FAD mice model Tg2576 (APPswe) compared with wild type C57BL/6 mice. Tg2576 and C57BL/6 males were fed WD or standard chow from the 3rd month of age and divided into 4-, 8-, 12- and 16-months old groups. Two brain structures were analyzed: the entorhinal cortex and hippocampus. Protein levels of p-IRS1(Ser616), p-Tau(Thr231) and APP were assessed by immunoblotting, while changes in neuronal location of p-Tau(Thr231) and Aβ formation were identified in brain sections by immunofluorescence.

Results: Under WD, early cerebral insulin resistance and altered p-Tau compartmentalization followed by Aβ formation were observed in wild-type mice. WD accelerated the onset of Aβ formation and p-Tau changes in Tg2576 mice, but independently of insulin signaling. The results showed differential sensitivity of hippocampal and cortical neurons to WD-related impairments.

Conclusions: Such findings are important for the development of personal strategies to prevention and therapies in FAD and SAD patients.

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P.1-40. Oral pathogens and proinflammatory environment amplify gingival fibroblast's ability to promote osteoclastogenesis

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Background: Osteoclasts are giant, multinucleated cells that mediate bone resorption and play an important role in pathological bone remodeling in many diseases, including periodontitis. Primary gingival fibroblasts (PHGF) significantly contribute to the development of periodontal inflammation but their contribution to bone resorption has not been thoroughly characterized. The goal of this work was to determine how PHGFs exposed to oral pathogens and inflammatory tissue environment influence osteoclastogenesis.

Material and methods: PHGFs were infected with *P. gingivalis* in the presence or absence of TNFα; after 48 hours the supernatants were collected, filtered, and concentrated. CD14+ monocytes isolated from human blood were cultured in PHGF-derived conditioned media for 19 days. Differentiated osteoclasts were fixed and stained for the osteoclast marker tartrate-resistant acid phosphatase (TRAP) and average areas of osteoclasts were calculated. The expression levels and production of osteoclastogenic mediators released by PHGFs were determined by ELISA and qPCR, respectively.

Results: Conditioned media from PHGFs were sufficient to stimulate osteoclast formation even in the absence of exogenous M-CSF and RANKL. Moreover, conditioned media from PHGFs exposed to *P. gingivalis* and/or TNFα were more potent in inducing osteoclast differentiation compared to media from untreated cells, with media from cells exposed both factors displaying the strongest osteoclastogenic potential. Consistently, the expression of osteoclast markers metalloproteinase-9 and cathepsin K was increased in monocytes treated with conditioned media from PHGFs infected with *P. gingivalis* in the presence or TNFα. Activated fibroblasts produce multiple factors that regulate osteoclastogenesis and we found that *P. gingivalis* and TNFα synergistically induce expression and/or production of IL-6 and prostaglandin E2 (PGE2) by PHGFs.

Conclusion: Our data indicate that in response of infection and inflammation PHGFs produce factors that promote osteoclastogenesis, which may contribute to bone loss in periodontitis patients. PGE2 and IL-6 are known to directly or indirectly regulate osteoclastogenesis and may be responsible for the observed effects. Ongoing experiments will verify the involvement if PGE2 and IL-6 osteoclastogenesis regulation by PHGFs.

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P.1-41. Quality of life before and for 12 months of observation after hemilaminectomy due to the stenosis of the lumbosacral (L/S) spine

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Background: Stenosis of the lumbosacral (L/S) spine (LSS) is a reduction in the space of the spinal canal, causing compression of nerve and vascular elements within the lumbar spine, and it occurs most often in the fifth and sixth decade of life. The most commonly used questionnaires for assessing quality of life include the medical outcomes study 36-item short-form health survey (SF-36), the Sickness Impact Profile (SIP), the World Health Organization Quality of Life (WHOQOL), and the Satisfaction with Life Scale (SWLS). The study aimed to evaluate the quality of life of patients with LSS before and during 12 months after hemilaminectomy.

Material and methods: The study group consisted of 96 patients with degenerative stenosis of the lumbosacral spine who qualified for surgical decompression We used the Satisfaction with Life Scale questionnaire at 0, 1, 6, and 12 months after treatment.

Results: In the group of patients treated surgically, during the first month of follow-up, most patients (61%) declared an improvement compared to the preoperative period. In 33% of the cases, patients subjectively reported no change. Only 6% of patients experienced a worsening of their health. At the 6-month follow-up, there was an increase in the percentage of patients who perceived an improvement in health (86%), with a simultaneous decrease in the percentage of patients declaring no improvement (11%) or worsening (3%). At the 12-month follow-up, 91% of the patients reported improved quality of life after hemilaminectomy. The percentage of patients declaring no improvement after the procedure also decreased (6%).

Conclusion: Surgical decompression of the L/S spinal canal in significantly increases life satisfaction for patients qualified for hemilaminectomy.

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P.1-42. Light as a chance to resensitize bacteria to antibiotics

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Background: One of the latest global reports from The Lancet (2022) revealed that in 2022, the third leading cause of death was infection associated with antimicrobial resistance. Therefore, new strategies to combat bacterial resistance to antibiotics are intensively studied. One of the promising strategies is antimicrobial photodynamic therapy (aPDI) based on the photosensitizer induced by a specific wavelength of light.

The bactericidal effect of aPDI can be multiplied by synergy with antibiotics. aPDI is a chance to re-sensitize bacteria to antibiotics by inducing morphological and functional damage in bacterial cells. Moreover, aPDI displays potential in biofilm eradication, while antibiotics alone have problems with penetration due to the biofilm structure.

Material and methods: The current study aimed to investigate whether combined therapy with clinically used antibiotics and aPDI (involving exogenously administered PS – rose bengal and green light, λ_{max} 515 nm) resensitize the clinical isolates of multidrug-resistant strain *Enterococcus faecalis* EU92 to antibiotics in planktonic and biofilm cultures. Their drug resistance profile and the synergy between aPDI and antibiotics were characterized according to the EUCAST standards, i.e. diffusion assays, MIC evaluation, checkerboard assay, and postantibiotic effect. Also, biofilm was formed using the approved methodology for flow-biofilm culture in a CDC reactor and treated with antibiotics and aPDI. The growth of biofilms and microbial survival after treatment was visualized using confocal microscopy.

Results: The obtained results confirmed that aPDI demonstrates a significant impact on *Enterococcus sp.* survival rate. Synergy was detected for aPDI in combination with gentamycin (GEN), streptomycin (STR), ciprofloxacin (CIP), daptomycin (DAP), tigecycline (TGC) and doxycycline (DOX). Combined treatment with STR and CIP also reduced bacterial load in biofilm culture by approx. 3-4 $\log_{10}$ CFU/cm^2 what was visualized on CLSM microscopic images.

Conclusions: aPDI has the potential to eradicate bacteria in planktonic and biofilm cultures. aPDI is also a promising tool for resensitizing multidrug-resistant bacteria strains to antibiotics.

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P.1-43. Spatial transcriptomics to dissect molecular signature of cardiac fibroblasts in experimental autoimmune myocarditis

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Background: Myocarditis is a severe inflammatory cardiac disease that can progress to dilated cardiomyopathy and heart failure. Experimental autoimmune myocarditis (EAM) represents an animal model of acute myocarditis, which is followed by the development of post-inflammatory cardiac fibrosis and systolic dysfunction. In this model, cardiac inflammation is characterized by the massive influx of myeloid infiltrates and is paralleled by the activation of extracellular matrix-producing cardiac fibroblasts.

Material and methods: 6–8-week-old BALB/c mice were immunized with αMyHC peptide together with Complete Freunds' Adjuvant to induce EAM. Heart tissues were isolated, embedded in OTC, sectioned into 10 µm-thick fragments, and placed on a 10x Genomics Visium Gene Expression Slide. Single-cell spatial libraries were prepared using 10x Genomics Visium protocols, and the sequencing was performed using Illumina Chemistry. Single-cell RNA sequencing data was analyzed using RStudio software.

Results: In our analysis, we included three biological time points: a healthy heart (d0), acute myocarditis (d19), and the resolution of inflammation (d25). Following sequencing, we analyzed 5911 spots under the tissue. We identified the main cell populations present in the mouse heart, including fibroblasts, immune cells, endothelial cells, smooth muscle cells, and cardiomyocytes. Subset analysis on cardiac fibroblasts resulted in the presence of four different subsets (FB1–FB4). Subset FB3 has a myofibroblastic signature defined by the expression of, among others, *Acta2*, *Postn*, *Vim*, *Col1a1* and *Col3a1*. This subset is transiently present at d19 of EAM. Spatial analysis of infiltrating immune cells characterized by the expression of, e.g., *Ly6c1* and *Lyz2* resulted in the presence of five subsets (INF1–INF5). The INF4 subset is present only at acute myocarditis phase (d19) and is spatiotemporally colocalized with the myofibroblastic subset (FB3).

Conclusions: Our data indicate that myofibroblasts and infiltrating immune cells colocalize within the tissue during acute phase of myocarditis. This may indicate active molecular interplay between inflammatory cells and cardiac fibroblasts.

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P1.44. Evaluation of the concentration of calcium in serum and intervertebral discs from patients with intervertebral disc degeneration

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Background: Intervertebral disc (IVD) degeneration is a complex and progressive process of disc aging. One of the most important causes of changes in the internal environment leading to intervertebral disc degeneration can be changes in the concentration of individual metal elements. The goal of this study was the assessment the concentrations of calcium in the serum and IVD samples obtained from patients with IVD degeneration.

Material and methods: The serum samples were obtained from 113 Caucasian patients who qualified for the microdiscectomy (study group) and 113 healthy volunteers (who, by current standards and guidelines, met the eligibility criteria for blood donors (control group). In turn, IVD samples were obtained by patients who qualified for the microdiscectomy (study group) and 81 human cadavers, during the post-mortem examination (control group). In the analyzed IVD samples, the content of calcium was determined using inductively coupled plasma – optical emission spectrometers (ICP-OES) AND acetylene-air flame atomization from serum samples.

Results: The concentration of calcium in serum samples was at the level 90.24 mg/L ± 8.77 mg/L vs. 91.50 mg/L ± 9.47 mg/L ($p>0.05$), whereas in IVD samples, the concentration of calcium was at level 1.93 g/kg ±2.74 g/kg vs. 0.13 g/kg ±0.03 g/kg ($p<0.05$).

Conclusions: It has been confirmed that the IVD degeneration process is associated with the calcification of the IVDs themselves without significantly affecting the serum calcium levels of patients with IVD degeneration.

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P.1.45. Polymeric nanocarriers for cardiovascular drug delivery: Development and initial evaluation of biocompatibility

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Background: Despite the ongoing development in invasive cardiovascular interventions and pharmacological therapies over the past 25 years, cardiovascular diseases continue to account for 31% of all deaths worldwide, amounting to 17.9 million deaths per year. Drug delivery systems may enable, e.g., longer circulation time of drugs or their targeted applications and are expected to improve therapeutic outcomes in the affected patients.

Material and methods: The polymeric nanocarriers (NCs) were prepared by nanoemulsion templating followed by the layer-by-layer technique. Two nano-core types were prepared: nanoemulsion droplets (AOT/PLL) and polymeric (polycaprolactone, PCL) nanoparticles. Selected cardiovascular drugs: carvedilol (CAR), lovastatin (LOV), and simvastatin (SIM) were encapsulated into the nano-cores. Such drug-loaded cores were further encapsulated into multilayer shells formed with biocompatible polyelectrolytes (poly-L-lysine hydrobromide (PLL), poly-L-glutamic acid sodium salt (PGA) and pegylated-PGA (PGA-g-PEG). All NCs were characterized by the determination of size, size distribution, zeta potential (DLS, ELS), and concentration (NTA). To investigate the biocompatibility of produced NCs, human umbilical vascular endothelial cells (HUVECs) were incubated with empty and drug-loaded NCs. Their growth was monitored for 48h using real-time cell analysis (xCelligence system). Nanocarrier concentrations were adapted to reported effective in vitro drug concentrations (5 µmol/L of CAR, 1 µmol/L LOV, and 1 µmol/L SIM).

Results: The average size of PEGylated and PGA-ended multilayer NCs was 100 – 150nm. Incubation of HUVECs with empty NCs (PCL-4W-PEG or PCL-2W-PGA) did not affect cell growth negatively, although PCL-4W-PEG nanocarriers were slightly better tolerated. Carvedilol alone and encapsulated in PCL-2W-PGA enhanced the growth of HUVECs. Cell growth and proliferation were not affected by LOV-containing NCs. Interestingly, in HUVECs treated with SIM-loaded NCs, improved growth as compared to control cells was observed in samples incubated with SIM-PCL-4W-PEG formulation.

Conclusions: The produced polymeric NCs are well tolerated at the concentrations corresponding to effective drug concentrations reported in vitro. Their respective effects on endothelial cell activation, migration, and response to inflammatory cytokines will be investigated in future studies.

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P.1.46. Biodistribution of polydopamine - a material for biomedical applications

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Background: Nanotechnology is undoubtedly one of the most dynamically developing fields of science and technology that has found application in medicine. Reducing the size of a selected material to the nanometer scale creates the possibility of numerous potential applications. Thanks to the strong adhesive properties, polydopamine nanoparticles (PDA-NPs) can surround any materials and the quinone groups present in the structure allow the attachment of metal ions, peptides, proteins, oligonucleotides. This makes it possible to obtain multi-purpose materials capable of preventing, diagnosing and treating diseases, with particular emphasis on cancer. Although the possibilities of using PDA-NPs are still growing, a full assessment of their **toxicological** profile has not been carried out.

Material and methods: The obtained materials in three sizes: ~50;~100;~200 nm and functionalized with metal ions (Fe3+, Mn2+ and Gd3+), were characterized using techniques (TEM, SEM, DLS, zeta potential, MRI imaging, ICP-OES). With the help of tests (Live/Dead and WST-1) their cytotoxicity profile was checked. The in vivo studies were preceded by the preparation and submission of an application to the Local Ethical Committee for Animal Experiments in Poznan (Resolution No. 16/2020). Studies involving a rat experimental model were carried out in the Laboratory of Experimental Animals of the Poznan University of Medical Sciences in Poland. MRI imaging was performed at the MRI Imaging Laboratory, NanoBioMedical Center, Adam Mickiewicz University in Poznan.

Results: Based on the obtained results, the relaxivity parameter (R1) was determined, which determines the effectiveness of contrasting a given substance, which were best in the case of PDA-Mn NPs: 109; 45; 42 [1/(mM*s)], for 50, 100 and 200 nm NPs, respectively. After examining the physicochemical properties of the obtained NPs, in vivo studies of PDA-NPs biodistribution were carried out using MRI imaging: before administration of PDA-NPs; immediately after administration; 1h; 7 days and 28 days after PDA-NPs administration.

Conclusions: The obtained results made it possible to assess the biodistribution of PDA-NPs and to establish certain criteria of toxicity (indication of critical organs, determination of toxic doses), thanks to which it will be possible to plan further stages of research.

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P.1-47. Generation of highly purified iPSC-derived cardiac derivatives for investigation of cardiovascular complications in rheumatoid arthritis

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Background: Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by joint involvement, in addition to several comorbidities, including cardiovascular (CV) complications. The aim of this study was to generate and characterize heart cells derivatives from induced pluripotent stem cells (iPSCs) of arthritic patient that may be of interest in understanding CV mechanisms in RA.

Material and methods: iPSCs derived from PBMC of RA patient were transduced with Sendai vectors. iPSCs pluripotency was assessed by IF, FACS and spontaneous iPSCs differentiation via embryoid bodies (EBs). Glycogen synthase kinase 3β induced iPSCs differentiation towards mesoderm. WNT inhibitor promoted iPSCs into the cardiomyocytes (CMs), VEGF towards endothelial cells (ECs) and inhibition of TGFβ signalling pathway (CFs). iPSC-CMs and iPSC-ECs derivates were purified by magnetic sorting (MACS), followed by IF, FACS characterizations and functional assessments.

Results: iPSCs demonstrated typical colony-like morphology and normal karyotype. Pluripotency was confirmed by the presence of OCT4, NANOG, TRA-1-81, TRA-1-60 and SSEA-4 and expression of specific ectoderm (NFH/PAX6), mesoderm (VIM/α-SMA) and endoderm (GATA4/SOX17) markers in spontaneously formed EBs. MACS-based sorting of heterogeneous iPSC-CMs population greatly improved purity of cells from 43.7% (unsorted CMs) to 95.3% of cTNT2 positive cardiopure cells. iPSC-ECs purification provided 97.9% of VE-cadherin and 95.3% of CD31 positive cells. iPSC-CMs displayed a cardiac-like morphology with spontaneous contractility and expression of cardiac specific markers (cTNT2, NKX2.5, HOPX, MYL2, MYH7, α-actinin). iPSC-ECs showed typical cobblestone-like morphology and the presence of CD31, VE-cadherin, vWF, and p-eNOS. Functionally, ECs angiogenic capacity was confirmed by Matrigel tube-formation and formation of sprouting sphere in response to VEGF stimulation. iPSC-CFs displayed spindle shaped morphology and the presence of VIM and COL1A1.

Conclusions: RA-derived iPSCs can be differentiated towards the mesodermal lineage allowing for generation of the principal cell types of the heart and subsequently to combine them in three-dimensional cardiac microtissues. It may offer a great potential to recapitulate the joint-heart interactions at a cellular level.

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P.1.48. Effect of dual-species biofilm on the membrane integrity of epithelial cells in a three-dimensional lung epithelial model

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Background: Colonization of the oral cavity by microorganisms can favour the inhabitation of pathogens in the respiratory tract and can lead to the development of aspiration pneumonia. *Candida albicans* create the natural mucosal microflora of human but it may cause life-threatening systemic infections, forming mixed biofilms with bacterial anaerobic periodontal pathogen – *Porphyromonas gingivalis*. The study aims to determine how the presence in the environment or direct invasion of mixed biofilms can affect the response and integrity of epithelial cells cultured in a 3D model.

Material and Methods: Human epithelial cells were cultured at air-liquid interface (ALI) cell culture for 4 weeks. Cells were treated for 24 h with supernatants received from mono- or dual-species biofilms or were in direct invasion with *C. albicans* (10^7 cells/mL) and *P. gingivalis* (10^8 cells/mL) or both. The permeability of the epithelium was detected using fluorescein isothiocyanate-dextran, and changes in the expression of E-cadherin and ZO-1 were detected by Western blot analysis. Human cells response was measured by IL-8 production, analyzed by the ELISA test.

Results: The results suggest that regardless of whether epithelial cells were stimulated with biofilm supernatant or colonized by pathogens, the integrity of human cells can be altered. The heterotypic biofilm can cause damage proteins involved in the formation of tight junctions (ZO-1) and adhesion junctions (E-cadherin), located on the cytoplasmic surface membrane of epithelial cells. Furthermore, the decrease in the expression level of ZO-1, and E-cadherin after contact with a mixed biofilm suggests that it may be more virulent compared to monospecies biofilms. The increased invasiveness of mixed biofilm appears to be confirmed by the decreased level of IL-8 production, which is significantly degraded after contact with the mixed biofilm containing bacterial proteolytic enzymes.

Conclusions: The results indicate the usefulness of the 3D model to study the impact of yeast-bacterial biofilm on the condition of lung epithelial cells. The presented data suggest that during bacterial and fungal infections, direct contact between the pathogen and the host plays a significant role, as well as components that can be secreted by pathogens to enable survival under unfavourable conditions.

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POSTER SESSIONS II

Preclinical Research

P.2-1. The effect of E-98, a novel histamine H_3 receptor antagonist, on anxiety behaviour and memory performance in mice model of neuropathic pain

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Background: Neuropathic pain (NP) is caused by the lesion or disease of somatosensory nervous system and may be characterized by both sensory and affective impairments. Indeed, NP patients manifest increased anxiety or memory impairment. In last years, there has been arising evidence for the involvement of histamine and its receptors in the modulation of pain. Moreover, histamine H_3 receptor (H_3R), has been proven to play a role in cognition and mood disorders. Taking into account these data, we aimed to assess the effect of the E-98, a novel H_3R antagonist, on anxiety and memory performance in mice model of NP.

Material and methods: Male Albino Swiss mice were divided into three groups: naïve, vehicle (veh) and E-98-CCI exposed. Veh and E-98 groups underwent chronic constriction injury (CCI) of the sciatic nerve. At day 7th after surgery, animals were chronically injected (i.p.) with veh and E-98 (10 mg/kg; twice daily) for 7 days. 14 days after CCI, mice were tested for anxiety behaviour in the open field (OF) test and for memory disturbances in the novel object recognition (NOR) test. After the tests, mice were sacrificed and the hippocampus was extracted for high-performance liquid chromatography (HPLC) analysis of monoamines (noradrenaline – NA; dopamine – DA; serotonin – 5-HT), and their metabolites (NM, DOPAC, HVA, 3-MT, 5-HIAA) levels.

Results: Neuropathic mice exhibited a lower number of rearings in OF test compared to naïve. In T1 phase (NOR test) no significant changes were observed between all groups, while in T2, naïve and E-98-treated animals explored novel object significantly longer. Veh-treated animals also spent more time exploring NO; however, the effect was fainter. The time spent on exploration of a familiar object (FO) in T2 was significantly higher in veh-treated group, compared to naïve and E-98 animals. In HPLC analysis, a lower amount of 3-MT was observed in the veh group, as compared to naïve animals.

Conclusions: The CCI procedure influenced animals' locomotor activity in OF and memory performance in NOR test and E-98 treatment reversed this effect. The E-98 did not affect hippocampal monoamine's level, suggesting a different and yet undefined mechanism of regulation of neuropathy-related affective impairment.

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P.2-2. Effect of doxorubicin on nerilysin activity in cardiac injury

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Background: Anthracyclines, the anti-cancer drugs, induce cardiotoxicity (AIC), which frequently results in heart failure with reduced ejection fraction (HFrEF). Increased interest in the function of neprilysin (NEP) in the development of cardiovascular illnesses due to the efficacy of a novel medication combination: angiotensin II type 1 receptor antagonist/neprilysin inhibitor in HFrEF patients is observed. The aim of the study was to evaluate the effect of doxorubicin (DOX) administration on the enzymatic activity of NEP within the left ventricle (LV).

Material and methods: The study was conducted on male SPRD rats divided onto 4 groups (n=8): 2 in acute AIC model (IaCON,IaDOX) and 2 in chronic AIC model (IbCON, IbDOX). Rats in the Ia groups were given one intraperitoneal (ip) injection of DOX (IaDOX) or NaCl (IaCON), then after 1 day, were sacrificed to collect hearts. Animals in the Ib groups were given four ip injections of DOX (IbDOX) or NaCl (IbCON) at weekly intervals, and were sacrificed 7 days after the last iniection, to collect hearts. Histopathological evaluation was performed on LV fragments by myocardial damage index (MDI). NEP protein levels were assessed by ELISA, enzymatic activity was evaluated by fluorometric assay. NEP mRNA expression was measured by RT-PCR.

Results: There was a significant reduction in the mean tissue NEP enzymatic activity in the LV in the IaDOXvsIaCON (95.92µU/mlvs119,51µU/m, $p<0.05$), which was not observed in the IbDOX. The significantly lower LV NEP protein level was observed in the IbDOXvsIbCON (1007.55ng/lvs1110,75ng/l, $p<0.05$), which was not observed in the IaDOX. No significant differences were observed between the control and experimental groups in terms of LV NEP mRNA expression in both models. There were statistically significant differences in histopathological assessment of MDI in IaDOXvsIaCON (16vs5.5, $p<0.01$) and IbDOXvsIbCON (14vs3, $p<0.01$). No statistically significant correlations were observed between NEP mRNA expression, tissue NEP activity, NEP protein levels, and histopathological MDI in both models.

Conclusions: NEP protein and its activity appear to influence the pathogenesis of AIC.

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P.2-3. MiR-200a-3p links neurodegenerative pathways to cell cycle control and shows promise as novel Alzheimer's disease biomarker and therapeutic target

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Background: Ageing-related, incurable neurodegenerative disorders such as Alzheimer's disease (AD) account for over 55 million dementia cases worldwide. AD pathogenesis is complex and unclear, involving various molecules in the brain and in peripheral tissues. Among them are genome-encoded microRNAs (miRNAs), 18-25 nucleotide non-coding RNAs that mediate post-transcriptional silencing of target mRNAs though binding to their complementary sequences within 3' UTR and causing degradation of the target transcript or reducing its translation. Some miRNAs can be released from cells and transmitted in the circulation. Mounting data show that miRNAs levels are deregulated both in AD brain and blood, opening the possibility for the identification of non-invasive, circulating AD biomarkers. Recently we identified hsa-miR-200a-3p as one of the most significantly upregulated miRNAs in blood plasma of patients at the early AD stage (doi: 10.18632/oncotarget.15109). The aim of the present study was to elucidate the function of miR-200a-3p in neurodegeneration.

Material and methods: Using literature search and bioinformatics (miRBase and TargetScan), we predicted target mRNAs of miR200a-3p. These targets were validated in human HEK293T cells transfected with miR-200a-3p mimic using luciferase assay, RT-qPCR, and immunoblotting.

Results: We demonstrated miR200a-3p direct interaction with the 3'UTR of β-secretase BACE1, the key enzyme of AD pathogenesis, and showed that miR-200a-3p downregulated endogenous BACE1 in dose-dependent manner, together with other transcripts encoding key proteins associated with neurodegeneration such as Ataxin-1, FUS, and PANK3. Moreover, cells transfected with miR200a-3p showed enhanced proliferation. Using cell-cycle related mRNA panel, we found that this effect can be attributed to the miR200a-3p-mediated upregulation of the cell cycle kinase 2 (CDK2) and downregulation of proapoptotic tumor suppressor TP53.

Conclusions: These data indicate that miR200a-3p orchestrates the regulation of neurodegeneration and of the cell cycle and apoptosis, and support the hypothesis that neurodegeneration may be a process similar to cancerogenesis but inversely regulated. As miR-200a-3p seems to play a role in AD pathomechanism, it can be developed as an early diagnostic biomarker and therapeutic target in AD.

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P.2-4. Investigating the therapeutic potential of targeting the histone acetylation system in *Porphyromonas gingivalis*-infected human macrophages

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Background: Modifying the histone acetylation system of the host is a well-established therapeutic approach for treating cancer; however, the use of epigenetic drugs targeting histone acetylation is not limited to cancer. In inflammatory diseases, such as periodontitis, they may also be useful. There are two ways to target acetylation: inhibition of histone deacetylases/acetyltransferases (HDAC/HAT) or disruption of the interaction between acetylated histones and transcription factors, which is facilitated by bromodomain-containing BET proteins. Our aim was to investigate how targeting these histone modifications affects human macrophages under inflammatory conditions associated with periodontitis.

Material and methods: Monocyte-derived macrophages (MDMs) were differentiated in medium supplemented with 50 ng/ml GM-CSF to induce the M1-like phenotype. MDMs were treated with BET inhibitors (BETi): 1 μM JQ1, Pan-HDACi: 0.5 μM ITF-2357 and HATi: 5 μM C646 for 1 h, followed by infection with *Porphyromonas gingivalis* at MOI 20 for 1, 5 or 24 h or its virulence factors: 100 ng/ml FimA and 100 ng/ml LPS. Gene expression was assessed by qPCR, protein production by ELISA and Western blot. The effect of the inhibitors on phagocytosis of pH-Rodo-labelled *P. gingivalis* was determined by flow cytometry.

Results: Infection with *P. gingivalis* upregulated several HDAC family members, including HDAC2-4;7-8, whereas the expression of HATs remained unchanged. Notably, stimulation with FimA upregulated several HATs and *HDAC3*, whereas LPS did not significantly alter HDAC and HAT transcript levels. Although several of the changes in mRNA expression were not detectable at the protein level, the use of inhibitors that interfere with histone acetylation proved useful in ameliorating inflammation. All tested compounds efficiently reduced the production of IL-6, IL-1β, TNFα, CCL2 and CCL5 with low cytotoxicity. In addition, these compounds do not have a major effect on the phagocytosis of *P. gingivalis* by MDMs.

Conclusions: Collectively, these results identify changes in HDAC expression as a potential new mechanism utilized by *P. gingivalis* to modulate the host immune responses, and suggest that therapies targeting HDACs, as well as other components of the acetylation system, may be therapeutically beneficial in periodontitis.

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P.2-5. Possible mechanisms underlying antidepressant action of SF-11 in the astroglial degeneration model of depression in rats

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Background: Our recent study has shown for the first time that a new brain penetrant Y2R antagonist SF-11 decreased the immobility time in the forced swim test (FST) after acute peripheral administration in naïve rats, indicating its antidepressant potential. In the present study, we evaluate the potential antidepressant properties of SF-11 in the astroglial degeneration model of depression with an emphasis on checking the possible mechanisms implicated in this antidepressant-like effect.

Material and methods: The model of depression was based on the loss of astrocytes in the medial prefrontal cortex (mPFC) through bilaterally administering the gliotoxin L-alpha-aminoadipic acid (L-AAA, 100 µg/2 µl) to Sprague-Dawley rats, once daily for 2 days.

SF-11 (10 mg/kg, *i.p.*) was administered once 1h before the FST on the 5th day of the experiment (72 h after the second L-AAA injection). The effects of L-AAA and SF-11 administered alone or in combined treatment were investigated using FST, Western blot technique, and immunohistochemical staining. *In vivo* microdialysis studies were performed on a separate group of freely moving rats, which received L-AAA unilaterally into the PFC (50 µg/1 µl), once daily for 2 days, whereas SF-11 (10 mg/kg, *i.p.*) was administered once, 72 h after the second L-AAA injection.

Results: L-AAA induced an increase in the immobility time of rats in the FST compared to the control group, indicating a depressive-like state. SF-11 reversed the L-AAA-increased duration of immobility in the FST with no effect on locomotor activity, indicating its antidepressive-like effect in this test. L-AAA diminished astrocyte densities in the rat mPFC, as demonstrated by Western blot analysis of GFAP protein level and by stereological quantification of GFAP-stained astrocytes. Immunoblotting and immunohistochemical analyses showed that SF-11 reversed the L-AAA-induced gliotoxic effect, indicating its glioprotective potential. Microdialysis study demonstrated that SF-11 decreased extracellular glutamate levels compared to basal value when administered alone as well as compared to basal value and control group when given into L-AAA-treated rats.

Conclusions: Our results demonstrate that the selective Y2R antagonist, SF-11, produced a rapid antidepressant-like effect, which may be connected with the inhibition of glutamatergic neurotransmission.

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P.2-6. The impact of β- carotene on proliferation, viability, and expression of EMT markers in prostate cancer cell lines

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Background: Among the wide variety of plant-derived phytochemicals, the group of carotenoids has continuously been studied to optimize their potential application in dietary intervention related to different diseases also the human prostate cancer (PCa). Carotenoids are involved in many intra- and intercellular signaling, cell growth and differentiation of prostate tissue. During PCa progression, epithelial cells can undergo epithelial-mesenchymal transition (EMT), characterized by morphological changes in their phenotype. Consumption of β-carotene (BC) has been shown to be a protective factor against the development of human PCa. However, the precise molecular mechanism of this phenomenon is not fully understood. Cellular models of PCa are well-established and give the opportunity to investigate them in a controlled environment. This may lead to the development of safe and efficient drugs against PCa.

Material and Methods: The study was carried out on human cell lines (ATCC) PC-3, LNCaP, 22Rv2, DU145, and RWPE-1 treated with different concentrations of BC. The cell lines were cultured according to the protocol. Each combination of cells and carotenoid was repeated 3-5 times. The proliferation and cytotoxicity of cells was determined by MTT and LDH Cytotoxicity Detection Kit. The expression of various proteins such as Snail, Twist, Zeb, E-, N-cadherins, AR, Bax, Bcl-2, and Akt were analyzed by Western Blot method. The concentration and uptake of BC by incubated cells was analyzed using HPLC assessment Shimadzu SCL-10AVP instrument (Japan), λ=450nm.

Results: The observed effect of changes in cell proliferation, cell migration, as well as expression of EMT transition proteins is dependent on the concentration of BC and the type of cells. It was shown that high as well as low doses of BC decrease the proliferation of prostate cancer cells in vitro but induced different answers in cell signaling (no cytotoxic effect was observed). BC is absorbed more efficiently from the medium by androgen-sensitive cells. We observed a decrease in the expression of vimentin, N-cadherin, and Snail in cancer lines and an increase in E-cad expression or re-expression in the PC-3 line.

Conclusions: Our results indicate that BC affects the signaling EMT pathways, participates in the inhibition of the development of aggressive forms of PC, and may alter the approach to therapeutic interventions.

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P.2-7. Proteomic Changes and Implications of Zinc Deficiency in the Prefrontal Cortex and Hippocampus

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Background: The prefrontal cortex (PFC) and hippocampus (Hip) are crucial brain regions involved in thinking and behaviour regulation. They are extensively studied in psychiatric illnesses, including depression, and are particularly affected by dietary zinc deficiency. Zinc is predominantly accumulated in the cortex, amygdala, and hippocampus, making these regions susceptible to zinc deficiency. Studies have shown that zinc deficiency increases the risk of major depression in humans and induces depressive-like behaviour in animal models. Coexisting factors in depressive spectrum disorders include metabolic alterations and mitochondrial dysfunctions. Mitochondria sequester a significant amount of cellular zinc, and the loss of zinc homeostasis may contribute to depression-related metabolic disturbances.

Methods: Mass spectrometry was used for protein analysis, and MaxQuant software was utilized for protein identification and quantification. Protein functional analysis was performed using MetaScape to identify enriched pathways. The proteomic data obtained were validated by assessing the activity of mitochondrial Complex I and Complex IV using colorimetric methods.

Results: Our study investigated proteomic changes in the PFC and Hip of rats on a zinc-deficient diet. We observed numerous protein alterations in these brain regions, indicating disruptions in zinc transport, mitochondrial function, axonal transport, calcium homeostasis, and ATP synthesis pathways. Specifically, we measured the activity of mitochondrial Complex I and Complex IV using colorimetric assay kits. We found a significant difference in Complex I activity in the PFC, suggesting a specific impact of zinc deficiency on Complex I function in this brain region. However, no significant difference was observed in Complex IV activity.

Conclusion: Our study demonstrates significant proteomic changes in the PFC and Hip of rats subjected to zinc dietary deprivation. These findings suggest disruptions in zinc transport, mitochondrial function, and ATP synthesis pathways. The assessment of mitochondrial Complex I activity specifically in the PFC validates these findings.

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P.2-8. Triple-acting 5-HT_6R/5-HT_3R antagonist/MAO-B reversible inhibitor superior over intepirdine in preventing and alleviating Aβ-induced memory decline

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Background: The emerging consensus about the multifactorial nature of Alzheimer's disease (AD) led to a change in drug discovery paradigms from single target to multitarget approach. Herein, we propose triple acting compounds targeting serotonin type 6 receptor (5-HT_6R), serotonin type 3 receptor (5-HT_3R) and monoamine oxidase type B (MAO-B), as a novel strategy for treating AD.

Materials and methods: Compound **PZ-1922** was identified from a 21-member library of structural analogs. Its affinity for 5-HT_6R and 5-HT_3Rs was determined in radioligand binding studies. The antagonist properties of **PZ-1922** at 5-HT_6R were tested in 1321N1 cells, followed by evaluation of its impact on HT_6R constitutive activity at G_s in NG108-15 cells. The antagonist properties at 5-HT_3R were determined using *ex vivo* method assessing guinea pig ileum contractility. The inhibitory activity at MAO-B was measured fluorometrically. The procognitive properties of **PZ-1922** were assessed in novel object recognition (NOR) task in scopolamine treated Sprague–Dawley rats and in T-maze test in Sprague–Dawley rats icv injected with oA$\beta_{25\text{-}35}$. The level of biomarkers disturbed by the oAβ icv injection was assessed by Western blot of hippocampus proteins.

Results: Application of the merged ligands concept identified the first-in-class triple acting 5-HT_6/5-HT_3R antagonist/MAO-B reversible inhibitor **PZ-1922**. It behaved as a neutral antagonist at 5-HT_6R-operated Gs signaling elicited by constitutively active 5-HT_6Rs. Furthermore, **PZ-1922** was brain penetrant and showed good bioavailability. **PZ-1922** reversed scopolamine-induced cognitive deficits in the NOR task and exhibited procognitive properties in rats icv injected with oA$\beta_{25\text{-}35}$ in both curative and preventive treatment paradigms. The data in the later model also demonstrated the superiority of **PZ-1922** over intepirdine and corroborated its improved capacity to prevent molecular and synaptic alterations in the hippocampus of Aβ injected rats.

Conclusions: The properties of **PZ-1922** demonstrate that simultaneous modulation of 5-HT_6R, 5-HT_3R and MAO-B is a promising approach for the development of new anti-AD agents.

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P.2-9. Selenoether derivatives of 1,3,5-triazine in search for novel class of neuroprotective agents of serotonin receptor 5-HT_6

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Background: In recent years 1,3,5-triazine derivatives were established as promising scaffold in search for novel neuroprotective agents. Here a new series of selenoether derivatives of 1,3,5-triazine with activity towards serotonin 5-HT_6 receptor (5-HT_6R) is reported.

Materials and methods: The compounds were obtained within 3-5 step synthesis, including Grignard reaction, and cyclic condensation. Affinities for 5-HT_6 and off-target receptors were tested in the radioligand binding assay, antioxidant capacity was measured by ability of compounds to reduce Mo(VI) to Mo(V) and neuroprotective activity was determined in in vitro assay on neuroblastoma SH-SY5Y cell line. Additionally in silico studies on possible binding mode between ligands and the 5-HT_6 receptor were performed.

Results: The majority of tested compounds showed significant affinity towards 5-HT_6R (K_i< 100 nM). The most promising compounds were selected for neuroprotection and antioxidation assays, in which they exhibited desired activity. In silico structure-activity relationship (SAR) analysis gave explanation of structural preference for stereoconfiguration and type of applied linker.

Conclusions: Results of both in vitro and in silico studies performed for the selenoether derivatives of 1,3,5-triazine indicated very potent 5-HT_6R affinities together with antioxidant and neuroprotective effects for three β-naphthyl derivatives with alkyl-branched linkers. Computer-aided SAR analysis performed provides area for further modifications. The obtained results gives hopes for discovery of novel class of neuroprotective 5-HT_6R agents

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P.2-10. Pharmacokinetic and behavioral profile for PPK32, the potent Se-containing 1,3,5-triazine 5-HT6 receptor agent with therapeutic future against Alzheimer's disease

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Background: The serotonin receptor 5-HT6 (5-HT6R), occurring almost exclusively in CNS, is a promising target in search for new drug against Alzheimer's disease (AD). However, none 5-HT6R ligand has passed clinical trials due to insufficient either pharmacokinetics or therapeutic effects in patients. In this study, the first potent 5-HT6R Se-antagonist PPK32 was investigated on procognitive and pharmacokinetic properties, in vivo and in vitro, respectively.

Materials and methods: In order to estimate procognitive properties, the effect of acute administration of PPK32 on MK-801-induced memory impairment, comparing to donepezil, in the Novel Object Recognition (NOR) test in rats was examined. Metabolic stability of PPK32 was investigated in vitro using rat liver microsomes. To assess pharmacokinetic profile and tissue penetration of PPK32 at a dose of 1 mg/kg, determined in behavioral studies, the male Wistar rats were used. The concentrations of PPK32 in serum and tissues were measured by a simple and sensitive reversed-phase HPLC/UV. PPK32 mean serum concentration-time profiles and key pharmacokinetic parameters in vivo were calculated based on noncompartmental approach.

Results: PPK32 turned out highly potent to reverse MK-801-induced memory disturbances at doses 0.3-3 mg/kg, i.e. lower than that demonstrated by donepezil (1 mg/kg). It also displayed an excellent metabolic stability to remain 97.13% intact. The tissue distribution profiles of PPK32 revealed that it was rapidly absorbed and readily diffused throughout all analyzed tissues, while its concentrations in brain were highest, reaching up to 52.94 and 54.87 ng/g after 15 and 30 min, respectively.

Conclusions: Based on pharmacodynamic and pharmacokinetic results obtained, the naphthylselenoether derivative of 1,3,5-triazine, PPK32, is very promising in search for innovative therapy of AD.

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P.2-11. Mass spectrometry analysis of proteomic changes in senescent aortic endothelial cells and their paracrine effects on adventitial fibroblasts

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Background: Aging is the most important risk factor for the development of cardiovascular diseases, mainly atherosclerosis. The age-associated increase in oxidative stress promotes the activation of cellular senescence. Senescent cells release plethora of factors commonly known as the senescence-associated secretory phenotype (SASP), which can modulate the normal function of the vascular wall. Vascular adventitia contains different cell types and their altered functioning and cross-talk can drive the cardiovascular diseases associated with neointima formation. However, the exact mechanisms of these processes are not yet well understood. Thus, the aim of this study was to characterize the oxidative stress-induced human aortic endothelial cells senescence and to evaluate the paracrine effects of these senescent endothelial cells on the primary cell type of the adventitia, the fibroblasts.

Material and methods: Human aortic endothelial cells (HAEC) were treated with hydrogen peroxide (75 µM concentration) to induce premature cell senescence. Then, human adventitial fibroblast cell line (hAdv cells) was treated with conditioned medium (CM) from senescent HAEC (HAEC-derived SASP). Lysates from both cell types were used for global proteomic analysis by liquid chromatography and tandem mass spectrometry.

Results: We identified the proteomic changes in oxidative stress-induced senescence and revealed that these prematurely senescent HAEC affect, in a paracrine manner, the proteome of adventitial fibroblasts. Majority of differentially expressed proteins in hAdv cells treated with CM from senescent HAEC were involved in uptake and metabolism of lipoproteins, mitophagy and ferroptosis. The proteomic changes indicated lower susceptibility of hAdv cells exposed to HAEC-derived SASP to ferroptosis. Based on the proteomic analysis we selected growth differentiation factor 15 (GDF-15) for its potential effect on erastin-induced ferroptosis of hAdv cells. Although, GDF-15, similarly to HAEC-derived SASP, affected some elements of the ferroptotic pathway in hAdv cells, it did not influence these cells susceptibility to erastin-induced cell death. **Conclusions:** Our findings can be of importance for potential therapeutic strategies targeting cell senescence or ferroptosis to alleviate neointima and atherosclerotic plaque formation.

Acknowledgements: This work was supported by grant ERA-CVD/MEND-AGE/6/2019 (NCBR).

P.2-12. The role of diacylglycerol lipase beta in an animal model of MIA-induced osteoarthritic pain

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Background: Osteoarthritis (OA) is a chronic joint disease, in which cartilage degenerates as a result of its mechanical and biochemical disturbances. The endocannabinoid system (ECS), a biological system linking pain transmission and immunological system possesses multiple novel targets for the treatment of pain. In particular, diacylglycerol lipase beta (DAGLβ), an enzyme responsible for the biosynthesis of the endocannabinoid 2-AG, is highly expressed on macrophages and has been implicated in inflammation and pain.

Material and methods: An intra-articular injection of MIA (0.56mg/10uL NaCl) was given to induce OA in rear right knee joint of male and female C57BL6/J (C57) mice as well as in DAGLβ wild type (WT) and DAGLβ knockout (KO) mice. The von Frey test was used to assess mechanical nociception on days 2, 10, 14, 21, 28, 31, 34, 37, 39 post-OA induction. On days 31-39 C57 mice were given an i.p. injection of vehicle, DAGLβ inhibitor (KT109; 40 mg/kg), MAGL inhibitor (MJN110; 5 mg/kg) or CB1 agonist (CP55,940 0.3 mg/kg).

Results: Female mice showed increased vulnerability to MIA injection than males and developed stronger allodynia even in the contralateral paw. DAGLβ-WT and DAGLβ-KO mice developed similar rates and magnitudes of MIA-induced allodynia, also KT109 did not reverse OA-induced hyperalgesia. This indicates, that neither deletion nor inhibition of DAGLβ reduces MIA-induced nociception in mice. Moreover, the MAGL inhibitor MJN110 did not affect MIA-induced nociception as well. In contrast, the CB1/CB2 receptor agonist CP55,940 reversed MIA-induced allodynia in male mice, but not female mice.

Conclusions: Female mice displayed considerably more sensitivity than male mice to the allodynic and toxic effects of MIA, which is consistent with the observation that women are more likely to suffer from OA than men. Genetic deletion or pharmacological inhibition of DAGLβ did not ameliorate MIA-induced allodynia in mice. These findings do not exclude the role of ECS in OA treatment and a potential role of other ECS elements for the modulation of pain, as CB1 and CB2 receptor agonists effectively reduce nociception in laboratory animal models of OA pain. However, the results indicate that DAGLβ may not be a viable target to treat OA pain.

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P.2-13. The fibroblast growth factor receptor 3 (FGFR3) as a potential marker for cancer stem cells in rhabdomyosarcoma

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Background: Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma in children, originating from undifferentiated embryonic mesenchymal cells. RMS comprises two histologic subtypes: embryonal (ERMS) and alveolar (ARMS). It is estimated that RMS accounts for 4.5% of all pediatric cancer cases. However, due to its rarity, research on RMS carcinogenesis is limited, and the causes and mechanisms of tumor development remain unclear.

Cancer stem cells (CSCs) are a small, specialized population of tumor cells with a high capacity for tumor initiation, invasion, metastasis and self-renewal, and are thus responsible for long-term tumor growth. Residual CSCs can recapitulate the original tumor.

The current treatments for RMS, mainly involving chemotherapy and radiotherapy, are often ineffective. Cancer stem cells may turn out to be a promising therapeutic target in development an innovative therapy against malignant RMS.

Material and methods: In our study, we examined established RMS cell lines: RH30, RH41 (alveolar subtype), and RD (embryonal subtype) using flow cytometry in 2D (monolayer) and 3D (sphere-formation assay) culture models to identify RMS-stem cells (RSCs).

Results: We attempted to identify rhabdomyosarcoma stem cells (RSCs) using cell markers. We evaluated expression of three potential markers: CD133 and FGFR3 (surface markers) and ALDH1 (Aldehyde Dehydrogenase 1, an intracellular marker). FGFR3 and ALDH1 were expressed by each cell line, while CD133 was only expressed by the RD line. Although we did not detect ALDH+/FGFR3+ cells, the analysis revealed a well-separated population of $FGFR3^+$ cells. Each rhabdomyosarcoma cell line contained a small population of $FGFR3^+$ cells (3-5%). We demonstrated that the addition of basic fibroblast growth factor (bFGF) maintained and enriched FGFR3-positive cells, which exhibited high self-renewal and proliferation capacity in the sphere-formation assay, confirmed also by the BrdU cell proliferation assay.

Conclusions: Our findings suggest that rhabdomyosarcoma cell lines include a minor subpopulation of FGFR-positive cells that can be maintained in long-term culture. Therefore, we propose FGFR3 as a potential marker for RMS-stem cells. Defining the CSCs markers will facilitate further studies towards a better understanding of RMS biology and the development of future anti-cancer therapies.

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P.2-14. Effect of cinnabarinic acid on schizophrenia-relevant behaviors in mice

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Background: Cinnabarinic acid (CA) is an endogenous, orthosteric agonist of the metabotropic glutamic acid receptor 4 (mGlu4). The hypothesis of hypofunction of the NMDA (N-methyl-D-aspartate) glutamate receptor assumes the occurrence of a disorder in glutamate signaling in the course of schizophrenia. At present, there is increasing focus on restoring proper glutamate signaling through modulation of mGlu receptor activity. mGlu4 receptor agonists and positive allosteric modulators (PAMs) have already shown efficacy in controlling schizophrenia-relevant behaviors in animal models. The study aims to investigate the influence of CA on schizophrenia-relevant behaviors in mice.

Material and methods: The research was carried out on male Albino Swiss mice. CA was administered once, intraperitoneally (i.p.) at a dose of 5 mg/kg b.w. Mice were tested in two schizophrenia-relevant behavioral tests: (1) prepulse inhibition test (PPI) to measure sensorimotor gating after CA injection, and (2) MK-801-induced locomotor activity to measure CA effect on MK-801-induced hyperlocomotion (0,3 mg/kg b.w.; i.p.). Additionally, the motor coordination of mice in the chimney test and locomotor activity were examined.

Results: No impairment of motor coordination was observed in mice in the chimney test following the administration of CA. In the locomotor activity test, CA increased the rest time, decreased vertical movements, and increased stereotypical movements in place. In the PPI test, CA failed to affect prepulse inhibition response and startle amplitude. CA affects MK-801-induced hyperlocomotion. CA decreased horizontal activity, total distance traveled, time in motion, and increased time at rest. Moreover, CA reduced the number of stereotypies, the time of stereotypy, and the number of repetitive behaviors.

Conclusions: Peripherally administered CA affects schizophrenia-relevant behaviors in mice.

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P.2-15. Modeling Parkinson's Disease with the Alpha-Synuclein Protein

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Background: Parkinson's disease (PD) is a slowly progressive neurodegenerative disease clinically characterized by progressive motor impairment. The main PD hallmarks include the loss of dopaminergic neurons in the nigrostriatal pathway and the development and accumulation of Lewy bodies composed of α-synuclein (α-Syn) aggregates. Alpha-synuclein is a key protein involved in PD pathology. Moreover, mutations in the gene SNCA (A53T) of alpha-synuclein cause familial forms of PD and are the basis of sporadic PD risk. α-Syn generates toxic oligomeric species that are stabilized by dopamine. The cytochrome P450 enzyme CYP2D6 metabolizes dopamine, neurosteroids and a variety of neuroactive drugs. Active CYP2D6 is expressed in the brain within the basal ganglia, a main area affected in PD. The CYP2D6 plays a key position in the metabolic regulation of anti-PD drugs and PD neurotoxins, supplying dopamine as well, so it can control disease risk along with protection.

Material and methods: SH-SY5Y cells are used in drug development and fundamental science - due to its human origin, catecholaminergic (dopaminergic) neuronal characteristics, and simplicity of maintenance. We designed and generated a lentiviral vector system that was needed to produce overexpression of hSNCA-A53T and hCYP2D6 genes in this line. Subsequently cell lines have been stabilized and protein overexpression confirmed by western blot, RT-PCR and phenotypically (antibiotic selection; hA53T-SNCA promotes cell proliferation).

Results: Here, we have generated a cellular model that reproduces PD overproduction of human α-Syn in cells combines with specific mutation (A53T) on SNCA gene. Additionally, we created overexpression of CYP2D6 in a such a line, and subsequently, a combination of α-Syn and CYP2D6 double overexpression was created to test the hypothesis that CYP2D6 can influence the metabolism of PD neurotoxins, anti-PD drugs, neurosteroid metabolism and the synthesis of dopamine, therefore regulating disease risk and offering protection.

Conclusions: In recent years, numerous models of PD based on α-Syn have been generated. In this work we deliver PD model utilizing a popular SH-SY5Y cell lines providing a qualified comprehensive molecular analysis tool for studies aimed at neuroprotection and PD disease modification, and influence of cytochrome P450.

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P.2-16. Assessment of psilocybin action in Wistar-Kyoto rats - behavioral tests followed by molecular analyses of BDNF-associated components

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Background: Psilocybin (PSY) improves the symptoms of depression after only a single treatment, and is efficient in treatment-resistant depression. The main goal of the present study was to assess PSY action in Wistar-Kyoto (WKY) rats, which endogenously display depressive-like behaviors.

Material and methods: PSY was administered to WKY or Wistar (WIS) rats i.p. once in a dose 0.3 mg/kg; control groups received saline. Novel object recognition (NOR) test was performed 4 h and 7 days after drug administration. Social interaction (SI) test was performed 2 and 8 days after drug administration. Forced swimming test (FST) was performed 9 and 23 days after drug administration. Serum BDNF level was assayed by ELISA and gene expression of *Bdnf*, *Ntrk2* and *Ngfr* in the prefrontal cortex (PCX) was measured by qRT-PCR.

Results: WKY rats demonstrated different behavioral patterns in comparison to WIS rats, i.e. lower discrimination coefficient in the NOR test; reduced social interactions in the SI test; and increased immobility time in the FST test. Concerning PSY action, in the NOR test there were no changes in PSY-treated groups in comparison to saline-treated groups in both rat strains. In the SI test, PSY treatment resulted in the increase of total social interaction time in WIS rats 2 days after drug administration, while in WKY rats the increased time of social interactions was significantly relevant 8 days after PSY treatment. In the FST test, the decrease in immobility time in WKY rats was observed 9 days after PSY treatment, while in WIS rats significant changes in the FST test were revealed as long as 3 weeks after single PSY administration. There were no significant changes in BDNF serum level after PSY treatment in either rat strains. However, some changes in gene expression of BDNF receptors in the PCX after PSY treatment were observed.

Conclusions: The obtained results indicated some differences in mood-related behaviors between WKY and WIS rat strains. PSY demonstrated its antidepressant and pro-social properties in both rat strains, although the time course of observed changes was distinct. Changes in the gene expression indicated the involvement of BDNF-related signaling in PSY action.

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P.2-17. GABA-A receptor alpha 2/3 subunit as a potential therapeutic target for essential tremor

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Background: Essential tremor (ET) is one of the most common movement disorders. Unfortunately, current ET pharmacotherapy is often ineffective, and the molecular basis of the disease is not yet fully understood. Harmaline is commonly used to model ET in animals. The main cause of harmaline tremor is abnormal activation of olivo-cerebellar glutamatergic climbing fibers, elevation of glutamate release in the cerebellum and an increase in complex spike discharges of GABAergic Purkinje cells. Our recent research suggests that potentiation of alpha 2/3 but not alpha 1 containing GABA-A receptors may attenuate harmaline tremor. The present study aimed to investigate the antitremor potential of two other GABA-A alpha 2/3 positive allosteric modulators, the hydrochloride salt of (5-(8-ethynyl-6-(pyridin-2-yl)-4H-benzo[f]imidazo[1,5-α][1,4]diazepin-3-yl)oxazole (KRM-II-81·HCl) and 2-(8-bromo-6-(pyridin-2-yl)-4H-benzo[f]imidazo[1,5-α][1,4]diazepin-3-yl)-4-ethyloxazole (KPP-III-34) in harmaline-induced model of ET.

Material and methods: KRM-II-81·HCl (3.2, 10 mg/kg ip) or KPP-III-34 (3.2, 10 mg/kg ip) were administered 30 min before harmaline (15 mg/kg ip). Immediately after harmaline injection animals were placed in Force Plate Actimeters for automatic 60 min measurement of tremor intensity and locomotor activity.

Results: Harmaline induced generalized tremor and had a characteristic biphasic effect on locomotion, initially weakening (0-30 min) and then increasing it (30-60 min). KRM-II-81·HCl (3.2, 10 mg/kg) and KPP-III-34 (10 mg/kg) significantly reduced harmaline-induced tremor between 30-60 min of measurement. Both compounds given alone lowered locomotor activity of rats in comparison to control but only in the first 30 min of measurement and had no effect on harmaline-induced changes in locomotion.

Conclusions: The obtained results confirm our previous observations and prove that stimulation of the α2/3 subunit of the GABA-A receptor can inhibit ET. Further, the studies with these two new GABA-A receptor potentiators opens the door to oral dosing given that both compounds are orally bioavailable. These findings encourage further research in this direction with the goal of developing new potential therapies for this prevalent neurological disorder without adequate medication options.

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P.2-18. Disulfiram effectively activates $G_{i/o}$-protein signaling by enhancing GDP/GTP exchange – possible relevance for opioid tolerance inhibition

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Background: Disulfiram - a potent acetaldehyde dehydrogenase inhibitor was originally intended for supporting abstinence in patients suffering from alcohol use disorder. However, experimental data has identified the enhancement of opioid-induced analgesia, reversal of opioid-induced tolerance, mitigation of opioid dependence and withdrawal as additional off-label properties of disulfiram. However, the molecular background of these phenomena has not yet been unraveled. This investigation strived to verify whether the modulatory effect of disulfiram on the opioid system stems from the enhancement of G protein signaling via modification of thiol residues.

Materials and methods: The [^{35}S]GTPγS assay was used to study G protein stimulation by disulfiram and its monomer metabolite – diethyldithiocarbamate in the presence or absence of GDP. The involvement of thiol residues was investigated with dithiothreitol. Modulation of morphine-induced G protein activation was also investigated.

Results: Disulfiram stimulated G protein activity with high effectiveness and micromolar potency by augmentatation of GDP binding, enhancement of GDP to GTP exchange and reduction in GTP affinity. Diethyldithiocarbamate failed to produce any effect. Disulfiram-induced G-protein stimulation was dose-dependently reversed by dithiothreitol. Additionally, disulfiram dose-dependently reduced the effectiveness of morphine in a naloxone-independent manner. On the other hand, a thiol alkylating agent – N-ethylmaleimide enhanced the effectiveness of G-protein stimulation by morphine.

Conclusions: Disulfiram enhances G-protein activation by forming disulfide bonds in the nucleotide binding pocket of the Gα subunit by facilitating GDP/GTP exchange and reduction of GTP binding. Additionally, disulfiram may interfere with morphine-induced conformational changes downstream from the μ-opioid receptor, thereby diminishing opioid effectiveness.

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P.2-19. Chronic administration of venlafaxine maintains field potentials and long-term potentiation in rat prefrontal cortex in ibotenic acid-lesioned, chronic mild stressed rats

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Background: Lesion of ibotenic acid to the ventral hippocampus (vHPC) resulted in attenuation of the monosynaptic glutamatergic transmission from the hippocampus to the prefrontal cortex (PFC). We postulate that such mechanism could be responsible for the non-responsiveness to antidepressants observed in treatment-resistant depression.

Material and methods: We investigate the effect of chronic administration of the serotonin and norepinephrine reuptake inhibitor - venlafaxine (VEN, 10 mg/kg, 5 weeks) on field potentials and long-term potentiation (LTP) evoked in rat prefrontal cortex by electrical stimuli. To attenuate vHPC-PFC transmission we used ibotenic acid (IBO, 5µg/0.5µl) bilateral lesion of vHPC in Wistar rats subjected to the chronic mild stress model of depression.

Results: As in previous studies, CMS procedure caused anhedonia measured by the decrease in sucrose consumption, which was further enhanced by IBO lesion in vHPC. This effect was reversed by chronic VEN treatment. VEN was equally effective against the effects of IBO in both control and stressed animals. In electrophysiological studies, the amplitude of extracellular field potentials recorded in cortical layer II/III was decreased by IBO lesion in stressed rats. Stress and IBO also reduced the magnitude of LTP. Both effects were partially reversed by VEN. Interestingly, IBO lesion in control rats caused an increase in Boltzmann amplitude and LTP recorded in prefrontal cortex, which was also normalized by VEN. Administration of IBO caused a 20% decrease in the number of neurons in the CA1 layer region of vHPC.

Conclusions: Lesion of the vHPC by IBO resulted in anhedonia, impaired glutamatergic transmission and long-term synaptic plasticity in prefrontal cortex, and cell loss in CA1 region of vHPC. Chronic administration of VEN was able to reverse anhedonia, and prevent the decrease in amplitude of glutamate-mediated field potentials evoked in layer II/III of rat prefrontal cortex and LTP, all caused by stress and IBO lesion in vHPC. Our results indicate, that chronic treatment with venlafaxine may restore glutamate transmission in the prefrontal cortex when connection between vHPC and mPFC is disturbed by IBO lesion. Such experimental conditions could mimic the impaired activity of vHPC-mPFC pathway observed in the resistance to antidepressant treatment.

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P.2-20. A compound screening platform for discovery of low-weight TrkB receptor agonists as potential treatment for depression

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Background: TrkB is a receptor with the tyrosine kinase activity and is modulated by the ligand binding which is BDNF. BDNF is a member of the neurotrophin family and acts as a key regulator of several neuro-anatomical processes. Targeting the BDNF-TrkB pathway by small molecular compounds may have antidepressant, procognitive and neuroprotective effects.

Methods: Compounds library was screened for interaction using medium throughput Microscale Thermophoresis (MST) method. First- line in vitro based screening of selected compound was performed by using SN56 cell line with stable TrkB expression (SN56 T48). Orthosteric activity was determined by using ELFI method (Enzyme-linked fixed cell immunoassay) In case of screening for allosteric modulators cells was treated in the combination of BDNF and molecules. Active compounds were further tested in differentiated SH-SY5Y cell line model and activation of downstream proteins (pAkt and pERK) was analyzed by Western blot. Preliminary selectivity testing Trk receptor family was performed using an appropriate inhibitor (K252a) and antagonist (ANA-12). Subsequently the ability of compounds to provoke TrkB dimerization and formation of the complex was monitored by native electrophoresis.

Results: 56 molecules with satisfactory affinity to TrkB receptor were identified after screening of 660 compounds. Kd value were determined for selected molecules, ranging from 2 to 200 µM. 2 molecules from 56 screened compounds induced TrkB phosphorylation in orthosteric agonist mode. One of them also positively modulated BDNF activity. 2 compounds increased phosphorylation of Akt and ERK proteins in a dose-dependent manner. Pretreatment with ANA-12 and K252a significantly decreased TrkB activity and downstream protein phosphorylation after TrkB agonist treatment. None of small molecular agonists provoked receptor dimerization.

Conclusion: A screening platform for selection of active and selective compounds- TrkB receptor agonists were established and proved its usability in search of novel molecules.

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P.2-21. Co-administration of low doses of psilocybin and mGluR2/3 antagonist LY341495 exerts antidepressant effects in mice

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Background: The discovery of the antidepressant properties of ketamine increased the scientific community's interest in exploring the potential role of the glutamatergic system in psychiatric illnesses. LY341495, the mGluR2/3 antagonist, has shown rapid antidepressant properties in preclinical studies. Concurrently clinical studies indicate a rapid and sustained antidepressant effect of psychedelic treatment. It is possible that psychedelics, including psilocybin, enhance glutamate transmission, which may be significant in the antidepressant mechanism of action. However, psychedelics produce many adverse effects. The combined administration of two antidepressant drugs in inactive doses may alleviate the side effects caused by one of the drugs and facilitates the action of the other drug. This study aimed to investigate the co-administration of psilocybin and LY341495 at antidepressant-inactive doses.

Material and methods: 7-week-old male mice of the C57/BJ/6 strain were used in the study. To verify the effect of the combined administration of psilocybin in a 0,5 mg/kg dose and LY341495 0.3 mg/kg (i.p.), we conducted standard screening tests used in the examination of antidepressant drugs: tail suspension test (TST), forced swim test (FST), and novelty suppression feeding test (NSFT). The effect of LY341495 on the hallucinogenic action of psilocybin was assessed by measuring head twitching response (HTR). For proteins levels changes in advance of cellular response, western blot analysis was performed for BDNF, ERK, and pERK proteins in the prefrontal cortex samples. Statistical analysis was performed using GraphPad Prism 8.0.1.

Results:The co-administration of 0.5 mg/kg psilocybin and 0.3 mg/kg LY341495 showed an antidepressant response in a TST performed 1 hour after administration, as manifested by a reduction in immobility time. In an NSFT conducted 24 hours after drug administration, the treatment reduced food latency time in the experimental arena. The antidepressant effect was maintained in the TST performed 72 hours and 7 days after administration.

Conclusions: Overall, this data indicates that the combined administration of inactive doses of psilocybin and LY341495 exhibits rapid antidepressant effects. However, further investigation needs to be done to reveal the exact possible mechanism of its joint action.

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P.2-22. Analgesic properties of a novel histamine H4 receptor antagonist in naïve and chronic constriction injury mice

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Background: Neuropathic pain is a pathology caused by lesions or diseases of the somatosensory nervous system. This type of pain is often refractory to standard therapies and become a growing public health problem. Histamine H4 receptor (H4R) has emerged as a promising drug target for pain alleviation. The aim of our study was to investigate the analgesic effects of a novel H4R antagonist, JSJ, in acute and neuropathic pain models in mice and its impact on microglia activation.

Methods: The studies were performed in naïve and chronic constriction injury (CCI)-exposed mice, representing acute and neuropathic pain models, respectively. The analgesic properties of a novel H4R antagonist, JSJ, on mechanical (von Frey) and thermal (cold plate, tail flick) stimuli were evaluated. JSJ was injected in a single (1, 10 and/or 20 mg/kg, i.p.) or chronic (20 mg/kg, i.p., twice daily for 7 days, starting from day 7th after CCI) regimen. We analysed the pattern of H4R expression within the spinal cord in naïve and neuropathic mice (Immunofluorescence assay). Moreover, we investigate the impact of JSJ (10 μM) on IL-1β, IL-6, and IL-10 levels (ELISA) in a microglial cell line (BV-2) after lipopolysaccharide (LPS) stimulation.

Results: Single injection of JSJ dose- and time-dependently reduced symptoms of neuropathic pain. Moreover, in the chronic treatment regimen, JSJ also reduced symptoms of neuropathy. In the acute pain model, JSJ diminished spinal nociceptive responses induced by thermal stimuli. Our immunofluorescence studies revealed that within the spinal cord, H4R colocalizes with microglia, astrocytes and neurons. In vitro studies demonstrated that JSJ significantly diminished levels of pro- (IL-1β, IL-6), but not anti-inflammatory (IL-10), factors in LPS-activated microglia.

Conclusions: Our studies bring the first evidence for the analgesic potency of novel H4R antagonist and its effect on microglia activation. We demonstrated the spinal expression of both neuronal H4R and its glial existence, highly implicating H4R role in neuropathic pain development. We hypothesize that the analgesic effects of H4R antagonists might be related to the modulation of glia-related neuroinflammation. Our studies shed new light on the role of the histaminergic system in neuropathic pain and propose H4R as a promising target for effective pain therapy.

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P.2-23. The mitochondria-targeted and slow-releasing H_2S donor, AP39 attenuates Concanavalin A-induced liver inflammation in mice

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Background: Autoimmune hepatitis (AIH) is a progressive necroinflammatory liver disease that can lead to liver cirrhosis and failure. The current standard treatment can have severe side effects and is not effective in 10-20% of cases. Therefore, there is a need for the development of new therapies. Recent findings suggest that liver mitochondria are the key element in the mechanism of hepatocellular death and liver injury in AIH. Hydrogen sulfide (H_2S) has been reported to exert anti-inflammatory and cytoprotective properties. Therefore, the aim of this study was to evaluate the hepatoprotective effect of AP39 – a mitochondria-targeted H_2S donor in Concanavalin A (Con A)-induced autoimmune hepatitis in mice.

Materials and Methods: Male Balb/c mice were divided into five groups: healthy control, Con A-induced hepatitis, Con A-induced hepatitis with pre-treatment of 100 or 1000 nmol/kg AP39, and Con A-induced hepatitis with post-treatment of 100 nmol/kg AP39. AP39 was administered intravenously 20 minutes before or 1 hour after the injection of Con A (15 mg/kg, *i.v.*). The animals were sacrificed at 15 hours after Con A dosing. The levels of pro-inflammatory cytokines in serum were measured by a multiplex Luminex™ method. The activity of liver enzymes such as ALT, AST, and LDH was determined using commercial kits from BIOMAXIMA. The expression of proteins involved in mitochondrial fission, fusion, and mitophagy was analyzed by Western blot.

Results: AP39 treatment significantly reduced the levels of IL-1β, IL-6, and TNF-α in serum of mice with Con A-induced hepatitis. Furthermore, AP39 decreased the activities of all studied liver enzymes that were increased after Con-A administration indicating its potency to reduce liver damage. The Western blot analysis revealed that AP39 increased the expression of the autophagy marker LC3B both in the whole liver homogenate and in the mitochondrial fraction. In addition, a decrease in the expression of PARKIN was observed. AP39 at the dose of 100 nmol/kg was found as an optimal. The pre-treatment regimen was more effective than the post-treatment compound administration.

Conclusions: AP39 decreases inflammation and liver damage which is probably related with mithophagy. AP39 has potential therapeutic effect in autoimmune liver diseases.

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P.2-24. Olfactory bulbectomy and zinc deficiency–induced changes in protein profiles in the hippocampus and prefrontal cortex of rats

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Background: Depression is a serious health problem but its pathophysiology is still unknown. Currently used antidepressants do not always provide the desired results and many patients suffer from treatment-resistant depression. Results from clinical studies suggest that zinc deficiency (ZnD) may be an important risk factor for depressive disorder as well as a factor decreasing the effect of antidepressants. The aim of the study was to investigate the expression of proteins level in the rodent model of depression and whether possible changes will be reversed by standard antidepressants.

Method: One group of rats was subjected to the bilateral removal of olfactory bulbs (OB). In control rats (Sham) the bulbs were left intact. 7 days following surgery, rats were fed a zinc-deficient diet (3mg Zn/kg) or zinc-adequate diet (50mg Zn/kg) for 3 weeks. Then, escitalopram (ESC), venlafaxine (VEN) 10 mg/kg, *i.p.* or combined ESC/VEN (inactive dose 1 mg/kg, *i.p.*) and zinc (5 mg/kg) treatment began. Following 3 weeks of drug administration the behaviour of rats was examined in behavioural tests. 24h after the test, rats were decapitated and PFC and Hp were collected for Western blot and Mass Spectrometry analysis.

Results: Mass Spectrometry analysis showed changes in the protein level involved in the development of depression such as CREB, BDNF, GluA1, PSD95 and in the zinc transporters protein level e.g.: Slc30a5, Slc30a1 Slc30a4, Slc30a7 in the PFC and Hp between control groups. Western blot analysis showed no changes after administration of antidepressants (ESC, VEN, Esc +Zn, Ven + Zn) compared to the control group ZnD + OB in PFC, while in Hp we observed only up-regulation trends in the above-mentioned proteins.

Conclusions: The OB + ZnD model induces more pro-depressive effects than either model alone. SSRIs seem to be not enough effective in this model which indicates that the OB + ZnD model may induce drug resistance in rats.

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P.2-25. Iron administered in the neonatal period changed the memory, mRNA BDNF expression and brain monoamine levels in adult Sprague-Dawley rats

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Background: Iron the most abundant metal in the brain, participates as a cofactor in key enzymatic reactions, and are relevant to neurotransmitter synthesis and degradation, dendritic arborization, and myelinization. Moreover, iron is a part of metaloproteins involved in oxygen transporter and energetic metabolism. However, it is widely recognized that iron, in the ferrous form, can react with hydrogen peroxide via the Fenton reaction producing the very reactive hydroxyl radica, which can demage cellular components including membrane lipids, proteins and DNA. Some available data indicated that concentration of iron in the brain progressively increases during the aging process in subjects, and it is selectively accumulates in the brains of patients suffering from neurodegenerative disorders. Iron-induced oxidative stress has been implicated in the pathogenesis of Alzheimer's, Parkinson's, and Huntington's disease, and others.

Material and methods: The aim of our study was to evaluate the influence of iron administered to rats (30 mg/kg, po) in the early postnatal brain development (p12-p14) on the recognition memory measure in the novel object recognition test, change in the social interaction and open field tests or in the BDNF mRNA expression, and in the monoamine levels in some brain structures involved in the regulation of working memory. The behavioral and biochemical tests were performed in adult p88-p92 rats.

Results: The present study indicated that iron administered to rats in the early postnatal brain development induced long-term deficits in the behavioral tests in the adult rats. In the social interaction test induced deficits in both parameters studied, it decreased the time of interactions and the number of episodes. In the novel object recognition test, evoked a decrease in memory retention. In addition, iron reduced exploratory activity (shortened the exploration time) in the open field test or decreased the expression of BDNF mRNA only in the hippocampus, and changed the brain monoamine levels in some studied brain structures.

Conclusions: The above data suggest the decreased BDNF mRNA expression by iron given in the neonatal period may play a role in iron-induced memory impairment in adult rats.

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P.2-26. Research towards an innovative molecule with potential action on Alzheimer's Disease

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Background: Alzheimer's Disease (AD) is one of the civilization diseases of the central nervous system (CNS), which is affecting a growing number of the population, especially the elderly. Nonetheless, no new synthetic drug that works in AD has been introduced to the pharmaceutical market for more than two decades. So far, various protein targets have been classified that may be involved in AD therapy, including serotonin 5-HT6 receptor (5-HT6R) and cyclin-dependent 5 kinase (CDK5). Examples of 1,3,5-triazines acting as both 5-HT6R ligands and CDK5 kinase inhibitors can be found in the literature. Thus, their simultaneous effect on above mentioned protein targets might possess a potential application in AD therapy.

Material and methods: The compounds were obtained in (i) one-pot reaction and (ii) three step synthesis including O-alkylation, cyclic condensation and Buchwald reaction. The compounds were designed on the basis of docking studies to improve the therapeutic effect on AD.

Results: With a molecular modelling support, a series of 14 innovative chemical compounds that combine in their structure the features of 5-HT6R ligands and the presence of a hinge region important in kinase molecular biology have been developed and tested on activity towards 5-HT6R and CDK5. Six compounds showed strong affinity towards 5-HT6R (Ki < 100 nM). Compound 10 exhibited the best affinity and a weak antagonistic effect against 5-HT6R (Ki = 6nM, pKb = 7.43), as well as moderate metabolic stability.

Conclusions: Unfortunately, despite promising results from molecular modelling, none of tested compounds displayed any effect on CDK5 kinase inhibition. Compound 10 has been chosen as a lead structure for further modifications including improving solubility and capturing the inhibitory effect on CDK5.

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P.2-27. Differences of mRNAs encoding blood-brain barrier-related proteins in the brain of stress-susceptible and stress-resilient mice

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Background: Stress is a non-specific reaction to threat or noxious stimuli, causing severe consequences for the organism. It is believed, that this process can trigger central nervous system disorders such as depression, anxiety, and post-traumatic stress disorder. More recently, experimental evidence suggest a critical role of stress in disruption of the blood-brain barrier (BBB), that regulates the movement of substances and blood-borne immune cells into the brain. It has been also known that individuals do not undergo stress and therefore are described as resilient. Such properties were exhibited by norepinephrine transporter knock-out mice (NET-KO) and SWR/J mice while C57BL/6J (WT) mice were susceptible to stress in behavioral tests. Also, differences between stress-resilient and stress-susceptible genotypes were proven at the molecular level. These findings led us to hypothesize that the BBB integrity should differentiate stress-resilient from stress-susceptible mice.

Material and methods: The expression of 29 mRNAs encoding BBB-related proteins in four brain regions, i.e. hippocampus (Hip), prefrontal cortex (PFCx), nucleus accumbens (NAcc) and cerebellum (Cer) was evaluated, with the use of Custom TaqMan Gene Expression Array Cards (Thermo Fisher Scientific).

Results: The most of mRNAs altered by stress were in the NAcc of NET-KO mice and in the PFCx of SWR/J mice. But most interesting were mRNAs changed only in the brain regions of WT mice or in both NET-KO and SWR/J mice under stress condition. The majority of altered mRNAs was in the Hip.

Conclusions: The proteins encoded by mRNAs which differentiated stress-susceptible from stress-resilient genotype can play a role in possible involvement of BBB in stress-resilience.

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P.2-28. Dual piperidine-based histamine H3 and sigma-1 receptor ligands in the treatment of nociceptive and neuropathic pain

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Background: The treatment of complex, multifactorial diseases by single target-oriented therapies rarely results in good efficacy. For this reason, the approach based on simultaneous modulation of multiple targets' activity captured the interest of the pharmaceutical industry and academia. Importantly, recent studies have shown that some clinically evaluated histamine H3 receptor antagonists (H3R) possess additional affinity at sigma-1 receptors (σ1R), which may play an important role in their pharmacology. Considering the clear relation between H3R and σ1R, along with the fact that dual-targeting compounds can lead to several improvements when compared to selective drugs, great effort should be made to develop such ligands for the treatment of various pain conditions.

Material and methods: In our study, we decided to combine chemical, biological and computational methods to reveal molecular properties responsible for histamine H3R and σ1R selective or dual-target binding of the studied compounds. Next, we designed a series of 16 new ligands and performed their pharmacological characterization using in vitro methods. Finally, lead compounds were tested in animal models of nociceptive and neuropathic pain.

Results: In a series of novel compounds, we selected three lead structures for further biological evaluation with high affinity at both H3R and σ1R. All of them did not demonstrate affinity at other histamine receptor subtypes and were characterized as potent H3R antagonists with the ability to penetrate across lipid membranes. Compound 12 showed a better safety profile than the other two tested compounds, hence we have selected this ligand for further analysis of its analgesic activity. The high potency of 12 in both, formalin- and capsaicin-induced pain indicated that this compound has the potential to attenuate neurogenic pain, regardless of the mechanism of its induction. Finally, we used two different models of neuropathic pain to test the influence of 12 on pain associated with neuronal tissue damage.

Conclusions: The obtained results strongly indicate that compound 12 can alleviate both chemotherapy-induced and sciatic nerve damage-driven neuropathic pain. This confirms its broad spectrum of analgesic activity based on the novel molecular mechanism.

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P.2-29. Effects of psilocybin on neurotransmitters release in the rat frontal cortex

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Background: Psilocybin is a psychedelic that exerts its effects by binding to various serotonin receptors. The main receptors through which psilocybin induces its effects are 5-HT2A and 5-HT1A. They are densely expressed on the pyramidal cells and GABAergic interneurons of the deep layers of the frontal cortex. The frontal cortex has broad connections to other parts of the brain and is responsible for the control of a wide array of functions, such as learning, motor activity, social behaviour, anxiety and mood. The aim of the study was to investigate whether administration of psilocybin influences levels of various neurotransmitters in the frontal cortex of a rat.

Material and methods: Wistar Han adult male rats were anaesthetized and microdialysis probes were placed in the frontal cortex according to the following coordinates (mm): AP +2.7, L +0.8, V −6.5. A week after implantation the microdialysis probes were connected to the syringe pump delivering artificial cerebrospinal fluid. After the initial washout period, five basal fractions were collected every 20 minutes. Then, a dose of psilocybin (0.1, 0.3 or 0.6 mg/kg in 2 ml/kg volume) was administered subcutaneously. The control group was administered with 0.9% NaCl solution. Then, fractions were collected every 20 minutes for 240 minutes. The microdialysis was performed in freely moving animals. The dialysates were analysed using HPLC with electrochemical detection.

Results: Acute administration of psilocybin influenced levels of all examined neurotransmitters in the frontal cortex of a rat. Extracellular levels of dopamine, serotonin and GABA were increased after administration of all analysed doses of psilocybin. Levels of acetylcholine were raised most potently after the administration of a 0.6 mg/kg dose of psilocybin, while noradrenaline was increased by doses of 0.1mg/kg and 0.6mg/kg, but decreased by the 0.3mg/kg dose. Glutamate extracellular levels were decreased by all of the analysed doses.

Conclusions: Psilocybin affects neurotransmission in the frontal cortex. The effects of psilocybin administration are dependent on a dose and result from relative stimulation of 5-HT2A and 5-HT1A serotonin receptors. The inhibitory control of GABAergic neurons overcomes excitatory effects of glutamate. The observed changes may play a role in anxiolytic and antidepressant effects of psilocybin.

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P.2-30. The contribution of the anti-apoptotic Bcl-2 family proteins to the deregulation of neutrophils in the presence of P. gingivalis devoid of gingipains

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Background: P. gingivalis is a keystone pathogen contributing to the development of periodontitis. It strongly induces an innate immune response by migration of PMNs to the site of infection leading to chronic inflammation. The study focused on determining the role of the main P. gingivalis virulence factors - gingipains in the deregulation of PMN viability and function to better understand the molecular mechanisms behind this process.

Materials and methods: The studies used two cell models, the HoxB8 neutrophil system and primary human neutrophils. They were stimulated with two wild-type P. gingivalis strains (W83 and ATCC33277) in the absence and presence of gingipain inhibitors and gingipain deletion mutant (ΔKΔRAB). The studies also included verification of an additive effect of the lack of gingipains and the inhibition of LPS-induced TLR-4 activation. The response of PMNs to infection was measured by their viability, production of ROS, NE activity, secretion of pro-inflammatory cytokines, and expression of anti-apoptotic proteins from the Bcl-2 family.

Results: Infection with P. gingivalis promotes prolonged PMN survival up to 48h, regulated by the anti-apoptotic Bcl-2 family proteins, and induces a pro-inflammatory response in periodontal tissues. Studies conducted on primary human PMNs have shown the significant role of gingipains in the deregulation of their functions, especially in ROS production and NE activity. Nevertheless, gingipains do not affect PMN viability and the development of a pro-inflammatory response. Stimulation of human PMNs with P. gingivalis-derived LPS Standard has significantly prolonged their viability which was also observed in the HoxB8 neutrophil system. However, unlike the mouse model, it is not involved in the induction of the neutrophil inflammatory response. Interestingly, studies conducted with polymyxin B indicate a significant reduction in the pro-inflammatory response of human PMNs. The results obtained for ΔKΔRAB in the presence of polymyxin B indicate no additive effect of the lack of gingipains and the inhibition of LPS-induced TLR-4 activation.

Conclusions: Understanding the deregulation of neutrophil functions and activity by gingipains both in the early and late stages of infection may contribute to the development of new and effective therapeutic strategies for periodontitis.

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Preclinical Research – Other themes

P.2-31. Interactive Simulation Technologies in Medical Therapy and Education

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Background: The ever-growing presence of information systems in diverse therapeutic approaches and medical education has become commonplace. A notable advancement in this field is the increasing utilisation of simulation technologies based on so called *game engines*, enabling highly sophisticated rendering of visual elements, real-time physics, advanced artificial intelligence, and direct compatibility with emerging display devices such as virtual and augmented reality HMDs, mobile devices, and wearables. As a result, the paradigm of *serious games* has found extensive applications in the realms of therapy and medical education.

Material and Methods: This paradigm encompasses the adoption of design mechanisms commonly employed in interactive entertainment systems for professional applications. A pivotal aspect of serious games lies in the employment of *mechanics*, which facilitate the dynamic adaptation of system states based on user behaviour and task performance quality. Furthermore, mechanics enable the modelling of various cognitive states of users, including emotions, engagement, immersion, as well as Knowledge, Skills, and Abilities. There exist systematic approaches, such as design patterns, that assist in the effective design of game mechanics. Additionally, dedicated systems enable the description and evaluation of mechanics and their interdependencies (balance), often represented structurally through graphs.

Results: In the comprehensive assessment of therapeutic and educational progress within these systems, intriguing modelling techniques like Bayesian Knowledge Tracing prove instrumental. We highlight the capabilities of game engines, outline the construction of an interactive application framework, demonstrate the utilisation of design patterns, and show the integration of artificial intelligence for both interaction and evaluation purposes.

Conclusion: Knowledge about the formal structure and existing frameworks for designing, implementing, and evaluating interactive simulations and applications can be crucial for certain medical specialists who will increasingly encounter such software in their work or be involved in its development.

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P.2-32. Differentiation of human induced pluripotent stem cells toward functional cardiomyocytes of psoriatic patient

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Background: Psoriasis (Ps) is a chronic inflammatory skin condition associated with increased cardiovascular (CV) risk compared to those without psoriasis. Generation of cardiomyocytes (CMs) from induced pluripotent stem cells (iPSCs) derived from Ps patients may provide a novel approach to investigate mechanisms by which cutaneous inflammation promotes CV complications in Ps.

Material and methods: PBMCs were isolated from whole blood of Ps patient with high disease activity and transduced with Sendai vectors to generate iPSCs. Characterization of the generated iPSC line was performed by staining for markers of pluripotency (IF and FACS) and differentiation of iPSCs via embryoid bodies (EBs) into cells originating from the three germ layers (IF and RT-PCR). iPSCs were subjected to differentiation towards CMs using small molecules regulating WNT pathway. Established iPSC-CMs were purified by magnetic cell sorting (MACS) and analyzed for the presence of CMs-associated markers (FACS and IF).

Results: Obtained iPSCs demonstrated typical colony-like morphology of undifferentiated cells, revealing the presence of pluripotent markers: SSEA-4 (99.5%), TRA-1-81(75.5%), OCT4 (98.9%), and Nanog (72.5%). The expression of specific markers for mesoderm (vimentin), ectoderm (NFH) and endoderm (SOX17) in the differentiated cells was demonstrated by IF and RT-PCR. Spontaneously beating CMs were obtained with the efficacy up to 85% of cardiac troponin T2 (cTNT2) positive cells. Magnetic separation of iPSC-CMs allowed to enrich positive selection of mature CMs. CMs demonstrated sarcomeric structure and the presence of CMs markers (cTNT2, α-actinin, Nkx2.5 and MYL2).

Conclusions: This is the first study demonstrating the generation of functional iPSC-CMs from the blood of Ps patient. Obtained iPSC-CMs may provide valuable model for investigation the mechanisms of cardiac dysfunctions in Ps.

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P.2-33. LPS-induced systemic inflammation promotes impaired insulin signaling leading to Alzheimer's-like changes in wild-type mice

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Background: One of the hypotheses for the ethiology of sporadic Alzheimer's disease (AD) focuses on systemic inflammation as a driving force of aging. Another known risk factor of AD is obesity and insulin resistance, known to be associated with the activation of pro-inflammatory cytokines.

The aim of this study was to verify the hypothesis that systemic inflammation induced by lipopolysaccharides (LPS) of Gram-negative bacteria may disrupt insulin signaling in the brain, leading to the development of neuropathological changes typical of AD. We tested this hypothesis in wild type mice by examining the LPS effect on the initiation and propagation of major neuropathological features of AD, such as amyloid-β (Aβ) and hyperphosphorylated tau protein (p-tau) pathologies that start from the entorhinal cortex and the hippocampus.

Material and methods: 3-months old male C57BL/6J mice were injected with LPS (0.5 mg/kg) for 3 days. Mice were then analyzed at 4, 8, 12 and 16 months of age for brain markers of: insulin resistance (p-IRS-1(Ser616)), p-tau pathology (p-tau (Thr231)) and amyloid pathology (APP levels) in the entorhinal cortex and hippocampus.

Results: The results revealed an increase in p-IRS levels from 4 months of age in both brain structures. In the entorhinal cortex an increase in p-Tau levels was observed in 8-month-old mice with no change in APP levels. Conversely, in the hippocampus, a decrease in levels of the full form of APP was observed in the 16-month-old group, with no change in p-Tau levels.

Conclusions: This results show that LPS, as an infectious, pro-inflammatory agent, can induce development of AD neuropathology features even in the absence of genetic alterations typical of AD. LPS induces insulin resistance in the brain, and then leads to an increase in tau phosphorylation in the entorhinal cortex in the early stage of the disease progression, followed by the amyloidopathy in the hippocampus.

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P.2-34. Platinum(II) and palladium(II) complexes as promising new anticancer agents

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Background: The discovery of cisplatin was made by Rosenberg, focused his attention due to the design of metal complexes that can be used in cancer chemotherapy. Particularly, an interest in palladium complexes is increasing, since they are closely related to their platinum analogues due to comparable physical and chemical properties

The aim of our study was to determine the cytotoxic effects of the newly synthesized palladium(II) and platinum(II) complexes with tris(2-carboxyethyl)phosphine on four canine cancer cells and red blood cells. In addition, the interaction between the platinum and palladium complexes and red blood cell membranes has been determined.

Material and methods: The effect of complexes on the proliferation of CLBL-1 (B-cell lymphoma), GL-1 (canine B-cell leukaemia), and two canine bladder cancer cells (K9-NK and K9-MP) was estimated by the ability of the cells to reduce the MTT. The cytotoxicity of complexes was also studied in relation to red blood cells. Using a microscope, the localization of compounds in RBCMs was determined based on changes in their shapes. In addition, the effect of the metal complexes on the properties of the hydrophilic and hydrophobic regions of the membrane was determined.

Results: The conducted studies indicated that both complexes show two-fold higher toxicity relative to CLBL-1 and GL-1 than two canine bladder cancer cells. On the other hand, the metal complexes in a wide range of concentrations do not cause hemolysis, but they change the shape of erythrocytes. The fluorimetric method showed that complexes used mainly affect the membranes hydrophilic regions.

Conclusions: To sum up, the results showed that complexes are able to decrease the viability of four cancer cell lines. In addition, both compounds are not toxic to erythrocytes and modify the biophysical properties of biological membranes. The newly synthesized complexes should be further investigated as they have great potential as drugs in cancer therapy.

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P.2-35. Glyceryl monooleate /glyceryl monolaurate lipid liquid crystalline nanoparticles as a potential miR-146a nanocarrier with cardioprotective properties

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Background: Ischemic heart disease (IHD) is a prevalent cardiovascular disease, and developing novel treatment approaches is of paramount importance. The application of miR-146a has emerged as a promising approach for IHD therapy, offering the potential to reduce cardiomyocyte apoptosis as well as vascular and coronary inflammatory responses. Glycerol monooleate (GMO) lipid liquid crystalline nanoparticles (LLCNPs) have been proposed as a promising vehicle to retain microRNA in blood vessels and deliver it to the cardiomyocytes. Furthermore, the successful addition of glycerol monolaurate (GML), which exhibit cardioprotective properties, to GMO LLCNPs may result in a multifunctional nanocarrier with both therapeutic and protective features for IHD treatment.

Methods: The optimal synthesis conditions for GMO/GML LLCNPs were determined by a central composite design (CCD). LLCNPs were prepared using a top-down approach. The internal structure of the resulting nanoparticles was evaluated over a range of temperatures using SAXS. Cryo-TEM was employed to obtain images of the nanoparticles. To assess the impact of the LLCNPs on cell viability (WST-1), a well-established model of *in vitro* cell culture was used.

Results: GMO/GML LLCNPs were successfully synthesized with GML comprising 11.67% of the total lipid mass. SAXS investigations confirmed the successful incorporation of GML into GMO LLCNPs, leading to the formation of structures exhibiting cubic inner symmetry. Cryo-TEM analysis provided further evidence of the presence of intricate internal structure. The WST-1 assay revealed no significant difference between GMO/GML and pure GMO LLCNPs.

Conclusions: The preliminary results present GMO/GML LLCNPs, a newly developed nanosystem with no harmful effect on cell viability. Future studies will focus on incorporating miR-146a with GMO/GML LLCNPs and evaluating the utility of the system for both therapeutic and cardioprotective applications in IHD treatment.

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P.2-36. Superparamagnetic Iron Oxide Nanoparticles as potent theranostic system: from circulating tumour cells capture to targeted drug delivery

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Background: Cancer is invincibly one of the leading causes of death worldwide. It has to be noted, that treatment of primary tumour and metastasis prevention are equally important and should both be addressed, preferably by the application of multimodal theranostic systems. SPIONs are known to be a valuable component of advanced drug delivery systems. They are recently extensively studied in the context of the circulating tumour cells (CTCs) capture and neutralization and magnetic drug delivery. Such systems are obtained by binding the specific protein or/and drug to the SPIONs' polymeric coating. The aim of our study was to prepare two types of colloidally stable SPIONs functionalized with selected drug and targeting ligand, enabling the capture of CTCs or delivery of chemotherapeutic to solid tumours.

Materials and methods: Two types of stabilized SPIONs were obtained by co-precipitation of iron salts with ammonia in the presence of cationic derivative of chitosan or hyaluronic acid. Pioglitazone and temozolomide were coupled with nanoparticles by forming complex with β-cyclodextrin covalently bound to SPION's polymeric coating via tosyl group. Targeting ligands (antibody or folic acid) were also covalently attached to the nanoparticles' surface. The average size and zeta potential of the resulting drug delivery system were characterized by DLS. ATR-FTIR and ^{1}H NMR spectra were also recorded, while magnetic properties of NPs were determined using Vibrating Sample Magnetometer and Möessbauer spectroscopy.

Results: Theranostic systems based on CCh/SPION and HA/SPION had an average hydrodynamic diameter of 204 nm or 228 nm, respectively, and were colloidally stable (zeta potential about 32 mV and -35 mV). Tosylation degree of β-CD was greater than 98 %. Conditions for drugs complexation with tosyl-β-cyclodextrin were established, along with the conditions of the targeting ligands coupling.

Conclusions: Nanoparticulate magnetic systems for potential use in CTCs capture and targeted drug delivery were obtained. Colloidal stability, suitable size and efficient drug loading make the proposed systems a promising tool for primary brain tumour treatment, allowing to decrease the metastatic potential of CTCs and reduce the severe side effects.

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P.2-37. Hydrogel flakes as an implantable formulation for local delivery of temozolomide

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Background: The most common malignant glial tumour is glioblastoma multiforme (GBL). Anti-GBL therapies remain palliative, however, they improve overall survival. A regimen with temozolomide (TMZ) chemotherapy followed by resection ensures the most promising results. Unfortunately, TMZ's therapeutic potential is hampered by severe systemic side effects after its intravenous or oral administration. Therefore, the studied herein systems for local delivery of TMZ seem to have a chance of meeting the requirements of more efficient treatment.

Material and methods: The proposed materials are in the form of lyophilized hydrogel flakes. The systems consist of biopolymeric matrices made of gelatin, chitosan, hyaluronic acid, and their derivatives as well as TMZ -polymeric conjugate introduced to the system in the form of particles or as a matrix component. Since along with different matrices compositions, distinct crosslinking methods were tested, the biopolymers were functionalized properly. The polymers' modifications as well as the TMZ-chitosan conjugation product were confirmed using NMR and FTIR spectroscopies. UV-Vis analysis provided quantitative information about TMZ. The hydrogel flakes were tested to meet the demands of the implantable products during degradation and swelling experiments.

Results: NMR and FTIR spectroscopies identified the products of biopolymers modifications and TMZ transformation. The obtained chitosan-TMZ conjugate exhibited drug-improved stability. Swelling ratio values for some of the lyophilized hydrogels revealed similarity to the commercially available neurosurgeon devices.

Conclusions: Our findings indicate that materials such as hydrogel flakes could be a good starting point for the development of a novel TMZ local delivery system with a more stable drug form and tuneable physicochemical properties. The proposed formulations' therapeutic effect will be validated *in vitro*.

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P.1-38. Potential of ID proteins as regulatory factors in rhabdomyosarcoma development

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Background: Rhabdomyosarcoma (RMS) is the most common type of pediatric soft tissue sarcoma among children. RMS is mesenchymal tumor that originates from an impaired differentiation of myogenic progenitors. Four ID inhibitors of differentiation belong to DNA-binding (ID) subfamily of helix-loop-helix (HLH) proteins. ID proteins play a fundamental role in the regulation of cellular proliferation and differentiation, as well as in tumorigenesis. The aim of our study was to investigate the novel, undescribed previously, roles of the selected ID proteins in RMS differentiation, growth and migration.

Materials and methods: Induced pluripotent stem cells (iPS) underwent myogenic differentiation towards skeletal muscles. RMS cells (alveolar: RH30, RH41 and embryonal: RD) were differentiated in DMEM low glucose medium with 2% horse serum. ID levels were diminished by transfection with siRNA. Gene expression levels were evaluated using quantitative real-time PCR. Proliferation and cell cycle were analyzed using BD Pharmingen APC BrDU Kit, whereas migratory capabilities were evaluated in a scratch assay and chemotaxis towards FBS and HGF was assessed using a modified Boyden's chamber with 8 µm pore polycarbonate membrane inserts.

Results: ID proteins were upregulated in myotubes formed after myogenic differentiation of iPS cells compared to undifferentiated iPS cells. Furthermore, differentiation of three different RMS cell lines diminished the levels of all ID inhibitors, which suggested their important roles in differentiation capabilities of the cells. Subsequently, ID2 and ID3 levels were downregulated by transfection with siRNA in RMS cells. ID2 silencing in RMS cells upregulated the levels of the selected myogenic regulatory factors, such as MYOD and inhibited proliferation of alveolar RMS cells. On the other hand, ID3 silencing, surprisingly, slightly increased the number of RMS proliferative cells in S phase and decreased the levels of MYOD. ID2 silencing exerted inhibitory effects on migration of RH41 alveolar RMS cells, whereas ID3 silencing diminished migration of three RMS cell lines in a scratch assay and decreased chemotaxis of RH30 cells towards FBS and HGF.

Conclusions: To conclude, ID2 and ID3 seemed to be opposite regulators of RMS proliferation and differentiation and similar regulators of migration *in vitro*.

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P.2-39. The proteomic analysis of *Streptococcus salivarius* extracellular vesicles and their impact on *Candida albicans* yeasts

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Background: *Streptococcus salivarius* is one of the probiotic bacteria exerting a positive impact on human health by supporting the natural microbiota and modulating the activity of the immune system. These functions are mostly associated with the release of molecules that demonstrate antimicrobial activity. *S. salivarius* produces nanometer-sized structures–extracellular vesicles (EVs)–containing different molecules, including proteins, phospholipids, and polysaccharides. Streptococcal EVs may be exploited to affect other microorganisms in the same colonized niche, including *Candida albicans* yeast-like fungus, an important human opportunistic pathogen.

Methods: *S. salivarius* EVs were isolated from bacterial colonies grown on BHI agar plates for 24 h at 37°C. The isolation procedure consisted of sequential centrifugation steps. The obtained EVs were visualized using a negative stained transmission electron microscopy. Proteins from *S. salivarius* EVs were identified with liquid chromatography-coupled tandem mass spectrometry. To analyze the effect of *S. salivarius* EVs on *C. albicans*, visualization with scanning electron microscopy, and counting colony-forming units (CFUs) were performed, after treatment of fungal cells with bacterial vesicles.

Results: The analysis of protein content of *S. salivarius* EVs showed the presence of 466 proteins with variable functions. The identified proteins were divided into 13 groups according to their biological role. Among these molecules, there were several proteins that may contribute to the probiotic function of *S. salivarius,* including surface adhesins, ABC transporters, penicillin-binding proteins, and bacteriocin immunity protein. Additionally, *C. albicans* growth was reduced after incubation of yeasts with bacterial EVs, and scanning electron microscopy visualization revealed partial destruction of fungal cells after this treatment.

Conclusions: The results obtained give an insight into the protein content of *S. salivarius* EVs and provide a better understanding of the interactions between these structures and yeast cells. Further studies are necessary for recognizing the mechanism of potential probiotic activity of vesicular molecules, and extended research on the effect of *S. salivarius* EVs on *C. albicans* is crucial to verify the potential usage of EVs in therapies against candidal infections.

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P.2-40. Superparamagnetic iron oxide nanoparticles surface-modified with succinylated chitosan for targeted anti-cancer therapy

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Background: Nanomedicine is a relatively new field of science that uses the achievements of nanotechnology in medicine. It found a broad application in oncology and contributed to the development of modern diagnostic and therapeutic methods. According to WHO data, one in six patients die from cancer. The efforts of scientists are focused on prevention, early diagnosis, and tumor removal or reduction of its size. Unfortunately, less attention is paid to the therapy of cancers in advanced stages, in which the metastasis is observed. This process accounts for around 90% of all cancer-related deaths. Metastases are largely caused by circulating tumor cells (CTCs) spreading throughout the body. Therefore, the aim of our research was to develop nanomaterials based on superparamagnetic iron oxide nanoparticles (SPIONs) stabilized with a N- succinylated chitosan (NSCh) surface modified with appropriately selected antibodies that will be used to capture CTCs.

Material and Methods: The N-succinylated chitosan derivative was characterized using ^{1}H NMR and ATR-FTIR spectroscopies, and the degree of substitution was determined using colorimetric ninhydrin test. The morphology, physicochemical, and magnetic properties of the NSCh-SPION were evaluated using dynamic light scattering (DLS), STEM microscopy, Mössbauer spectroscopy and magnetometry. The attachment of the antibody was confirmed by immunostaining using fluorescence spectroscopy.

Results: The results of spectroscopic analyses confirmed successful synthesis of NSCh. The obtained chitosan derivative has shown to have a high degree of substitution (55,49 %) and an excellent solubility in water. The obtained NSCh-SPION nanoparticles were a spherical, colloidally stable, and exhibited excellent magnetic properties. During the study, the formation of the NSCh-SPION-antibody system was also confirmed.

Conclusion: We have synthetized and optimised a targeting system based on SPION nanoparticles designed for CTCs capture. The physicochemical and magnetic properties of the obtained system were determined. Preliminary biological studies were also performed.

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P2-41. Synergistic activation of gingival fibroblasts by *Fusobacterium nucleatum* and interferon-γ as a new driver of chronic inflammation in periodontitis

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Background: Periodontitis is a chronic inflammatory disease of the gum that affects around 10% of the human population and is driven by microbial imbalance. *Fusobacterium nucleatum* (*Fn*) participates in disease development through biofilm creation, bacterial dysbiosis, and stimulation of host inflammatory responses. Gingival fibroblasts (GFs) are important sentinel cells that interact with oral bacteria and inflammatory tissue environment, promoting recruitment of immune cells to the site of inflammation in periodontitis and therefore represent a possible focal point for developing new treatment strategies for periodontal inflammation.

The goal of this study was to characterize how the interplay between the proinflammatory mediator interferon gamma (IFNγ), elevated levels of which are detected in periodontitis patients, and *Fn* affects GF inflammatory responses.

Materials and methods: Primary GFs from healthy donors were simultaneously infected with Fn and stimulated with IFNγ. RNA sequencing was performed to screen for potential targets for further study. Western blot analysis was used to analyse signalling pathway activation. ELISA was used to confirm amplified protein secretion by GFs.

Results: RNA-seq revealed amplified expression of several CXC family chemokines, colony stimulating factors, interleukins, TNF, and components of the MAPK, NFκB, and JAK-STAT pathways in GFs stimulated with IFNγ in the presence of *Fn*. These transcriptional effects translated into significant amplification of IFNγ-induced CXCL9 and CXCL10 production by GFs infected with *Fn*. Western blot analysis identified synergetic activation of STAT proteins, including STAT1, STAT3 and STAT5, in GFs exposed to both IFNγ and *Fn*. The observed amplification of STAT signaling was likely mediated by the cross-talk with MAPK and NFκB signaling, and required secretion of secondary mediator(s) by GFs.

Conclusions: We identify synergistic activation of GFs by bacterial infection and IFNγ as a potential new mechanism responsible for perpetuation of chronic inflammation in periodontitis.

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P2.42. Effect of calcitriol and tacalcitol on gene and protein expression associated with Th17 cell differentiation in metastatic mammary gland cancer model

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Background: Breast cancer has the highest rate of disease and death in the world among women with cancer. Previous studies have shown that calcitriol and its derivatives in young 4T1 tumor-bearing mice increased the expression of osteopontin, which induced Th17 lymphocyte differentiation, leading to the release of pro-inflammatory IL-17. In old mice the opposite effect was observed. Osteopontin together with calcitriol and tacalcitol modulates T lymphocytes, regulates migration, adhesion and activation of inflammatory cells. The aim of the study was to evaluate the effect of the active form of vitamin D - calcitriol and its less toxic analogue - tacalcitol on Th17 lymphocytes in breast cancer and to investigate the role of osteopontin in this process.

Material and methods: Mice aged 6-8 weeks and 40 weeks (ovariectomized) inoculated with 4T1 tumor cells were oral administered calcitriol and tacalcitol for 24 days. After a 24-day in vivo experiment, a spleen was collected, CD4+ cells were magnetically isolated and analysed for genes expression important for Th17 differentiation using Real time PCR (*Il-17a, RorC, Stat3, RorA* genes) and automated proteins detection to investigate activation of signal pathways involved in Th17 differentiation (ERK, JNK, p38 pathways).

Results: Real time PCR analyses showed that *RORc* gene was overexpressed in premenopausal mice as a result of treatment with calcitriol and tacalcitol. A similar effect was observed for *IL17a*, but it was not statistically significant. For postmenopausal mice, the opposite effect was observed, but it was also not statistically significant. Analysis of MAP signaling pathway proteins showed that ERK and JNK pathway proteins were activated in postmenopausal mice treated with calcitriol or tacalcitol, whereas in premenopausal mice there were no significant differences in the activation of these proteins as a result of treatment.

Conclusions: The results indicate the need for further studies in order to better understand the modulation mechanism of Th17 cell differentiation due to calcitriol and tacalcitol treatment in pre- and postmenopausal mice.

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P.2.43. The influence of the contact with host proteins and neutrophils on the fungal proteome of biofilm of *Candida albicans* and *Porphyromonas gingivalis*

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Background: *Candida albicans,* a fungal pathogen colonizing mucous areas of the human body, can cause candidiasis in the host with weakened immunity. It has the ability to form biofilms with other microorganisms, which exist in the oral cavity, as well as with periodontal bacterium *Porphyromonas gingivalis*. Presented studies show impact of host's organism (considered as serum or neutrophils) influence on changes occurring in fungal proteome secreted to biofilm matrix.

Material and methods: Mixed fungal-bacterial biofilms were formed by 10^6/ml *C. albicans* ATCC® 10231™ cells and 10^7/ml *P. gingivalis* cells strains W83 and ΔKΔRAB (lacking the gingipains R and K genes). Cells were inoculated for 48 hours in 37°C in RPMI 1640 with addition of 10% fetal bovine serum (FBS) or with 10^6/ml of neutrophils isolated from EDTA-treated whole-blood samples obtained from healthy donors. Biofilm matrix was harvested by sonication followed by several centrifugations, lyophilized, and then resuspended in extrapure H_2O. The LC-MS/MS analysis was performed on a Q Exactive mass spectrometer coupled with a nanoHPLC (UltiMate 3000 RSLCnano System). Obtained data were processed with the Proteome Discoverer platform (v.1.4; Thermo Fisher Scientific) and searched using an in-house MASCOT server (v.2.5.1; Matrix Science) against protein sequences of Fungi from the SwissProt database.

Results: Presence of FBS or neutrophils alters fungal proteome secreted to matrix of biofilm. Serum stimulates the secretion of proteins involved in *Candida* virulence in contact with the most virulent strain of *P. gingivalis* – W83. The percentage content of proteins important for carbohydrate metabolism (including glycan biosynthesis and degradation) is increased under these pathological conditions, as well as for other proteins involved in different pivotal metabolic pathways (such as energy or lipid metabolism). Furthermore obtained results show incorporation of human proteins from serum or neutrophils to biofilm matrix.

Conclusions: The coexistence of fungi and bacteria in mixed biofilm, together with factors present in the host organism and secreted as a result of infection, alters the proteome of the biofilm matrix, leading to changes in its structure and properties, as well as interfering with the possibilities of treating mixed-species infections.

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P.2.44. Four peptides from milk fermented with kombucha cultures as potential antifungal agents and their coordination with copper and zinc ions

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Background: Candida auris is an emerging multidrug-resistant Candida species that poses a serious global health threat. A good model for this fungus is Candida albicans.

We selected four peptides from those identified in kombucha-treated milk that have already shown potent antihypertensive activity to screen for their additional bioactivity. Their interactions with the Cu(II) and Zn(II) metal ions were also investigated. In addition, the antifungal activity of these peptides was tested against C. albicans, an opportunistic human pathogen that causes mucosal and systemic infections in susceptible individuals.

Material and methods: ITC and susceptibility tests on fungi were performed at a pH of about 6 to mimic the pH of human skin. The tests on Candida albicans were performed using the dilution sensitivity test method in Sabouraud dextrose liquid medium. Metal-peptide interaction affinity and stoichiometry were studied by isothermal titration microcalorimetry (ITC), MS, potentiometry and CD - and UV-vis spectroscopy.

Results: All peptides studied: AVPQEVLNENLLR, YLQGSNLVVPLTDD, KFKGFVEPFPAVE and FVAPEPFVFGKEK bind Cu(II) with moderate affinity and 1:1 stoichiometry. The binding of zinc at the same pH (6) is much weaker. The ITC results show that all reactions are entropy-driven. Antifungal susceptibility tests of Candida albicans have shown that only the first peptide has a weak antifungal effect, both for free and copper-bound complexes.

Conclusions: The antifungal tests should be repeated for AVPQEVLNENLLR with a higher concentration of the peptide. Antimicrobial testing can also be done for bacteria that cause skin disorders. It can then be used for skin tests to treat bacterial and fungal diseases.

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Clinical Research

P.2-45. Recovery prediction for brain tumor with machine learning and augmented reality

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Background: Virtual reality (VR) technology has become increasingly prevalent in the field of surgical planning due to its ability to provide a detailed visualization of anatomical structures. Machine learning (ML) has also shown promise in predicting the rewiring of the brain. This study shows the usefulness of a predictive ML-based system to guess the rewiring of the brain as structural brain connectome. The results are provided by a VR system to be used during surgical planning.

Material and Methods: A predictive system based on machine learning and healthy anatomical information, we predict structural rearrangement after surgery given the preoperative brain network. A VR system was was developed using Unity 3D to visualize the results. The system was designed to allow surgeons to interact with 3D models of patient-specific anatomical structures and to use ML algorithms to predict the rewiring of the brain. The ML algorithms were trained on data from previous surgeries and used to make predictions about the rewiring that would occur during the current surgery.

Results: The VR system combined with ML algorithms provided accurate predictions of the rewiring that occurred during the surgeries. The system also allowed surgeons to interact with the 3D models and visualize the anatomical structures in a more detailed and intuitive way.

Conclusions: The combination of VR technology and ML algorithms shows great promise in improving surgical planning and predicting the rewiring of the brain. The use of a VR system provides surgeons with a more immersive and interactive experience, which can aid in the decision-making process. Additionally, the use of ML algorithms can help to predict the outcomes of surgeries more accurately, allowing for better planning and preparation. We foresee that the proposed tool can at least be used for educational purposes or simulation before a real surgery. Future developments include a similar approach for deep brain simulation interventions.

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P.2-46. Application of panel of protein endothelial dysfunction to study endotheliopathy of systemic and cerebral circulation in schizophrenia

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Background: Schizophrenia is a mental disorder with a high risk of premature death. Despite many studies on schizophrenia, the mechanisms that contribute to the development of endothelial dysfunction in this disorder are still not fully understood.

Material and methods: So far, it has been developed microLC/MS/MS based targeted proteomic analysis focused on the detection of various aspects of endothelial dysfunction such as glycocalyx disruption, endothelial inflammation, increased endothelial permeability, disturbed hemostasis and other processes related to the dysfunction of the vascular endothelium. We previously applied this methodology to study in *e.g.* mouse models of fatty liver caused by high-fat diet, atherosclerosis, endotoxemia induced by intraperitoneal administration of lipopolysaccharide and metastatic breast cancer (4T1), and in patients with obstructive sleep apnea. Here we extend this methodology to adapt to analyse the endothelial phenotype in patients with acute psychosis, adding to the panel the following biomarkers: thrombomodulin and myelin-associated glycoprotein. The aim of this study is therefore to analyse whether panel of biomarkers of endothelial dysfunction including eighteen selected biomarkers [SDC-1, sVCAM-1, sICAM-1, sE-sel, Angpt-1, Angpt-2, sFLT-1, sTie-2, vWF, t-PA, PAI-1, THBS-1, TAFI, TM, ADM, ADN, ANXA5 and MAG] could identify phenotype of endothelial dysfunction in young patients with acute psychosis. Additionally, it is planned to assessed the bioavailability of NO by measuring the concentration of its metabolites in plasma.

Results: Our results indicate that endothelial dysfunction in acute psychosis patients associated with the fall in NO was not associated with clear-cut alterations in the biomarkers of endothelial dysfunction measured in the panel. However, in young patients with acute psychosis some changes in selected biomarkers response were noted as compared to pattern in in healthy volunteers suggesting altered endothelial phenotype (*e.g.* sVCAM-1, TAFI, THBS-1), but it remains to be established what is the significance of these changes.

Conclusions: MicroLC/MS-MRM method enables to characterize the phenotype of endothelium in young patients with acute psychosis based on panel of biomarkers measured in plasma.

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Center for Development of New Pharmacotherapies of Central Nervous System Disorders.

Creation of a technologically advanced Center for the Development of New Pharmacotherapies of the Central Nervous System (acronym: CEPHARES) at the Maj Institute of Pharmacology, is a response to the growing and still unsatisfied therapeutic needs in the field of depression, anxiety, schizophrenia, drug addiction and neurodegenerative diseases.

The mission of CEPHARES will be the implementation of advanced, interdisciplinary research using the cutting-edge equipment infrastructure, closely correlated with the path of development of candidates for innovative drugs based on the use of sophisticated methodology. These will include chemo- and bioinformatics, medicinal chemistry, organic synthesis, modern biochemistry, molecular biology, electrophysiology, transgenics and - what is a traditional methodological background of IP PAS – a wide spectrum of pharmacological methods for behavioral phenotyping of Central Nervous System diseases animal models.

CEPHARES is planned to be opened in 2024, and – apart from being a platform for cooperation with pharmaceutical industry – will serve also as a Core Facility for scientific community of the Institute and our collaborators. Researchers will gain access to fully equipped laboratories located in brand new facilities, grouped in 5 thematic modules:

- CF1 – bioinformatics (laboratories of in silico modelling and computational genomics);
- CF2 – drug chemistry (laboratories of optimalisation and drug synthesis, analytical chemistry);
- CF3 – bioanalysis (laboratories of high-throughput screening, proteomics and mass spectrometry, pharmacokinetics and drug metabolism);
- CF4 – drug mechanisms (laboratories of ex vivo / in vitro research, imaging);
- CF5 – behavioral models (laboratories of transgenic animals and behavioral phenotyping).

Examples of equipment already purchased and being subject to tests in the phase of implementation are: Amersham Typhoon bioanalyser, Opera Phoenix High-Content Screening System, Nvidia AI server, Seahorse XF metabolic analyser, DIVE8 multiphoton microscope, TIMS TOF Pro2/PASEF mass spectrometry, Protein SimpleJess analyser and many more.

We are striving to find new and creative ways to continue our work right after the CEPHARES will be launched and carry forth our great collaborations. We look forward to connect and stay connected to you virtually: https://www.facebook.com/CEPHARES/ www.cephares.pl.

Centre for the Development of Therapies for Civilization and Age-Related Diseases (CDT -CARD) – a new R&D division of the Jagiellonian University Medical College

The Centre for the Development of Therapies for Civilization and Age-Related Diseases (CDT-CARD) is an interdisciplinary and innovative research center focused on the design and testing of novel treatments for civilization and age-related diseases, specifically targeting preclinical trials. CDT-CARD aims to establish a strong connection between basic, preclinical, and clinical research, while incorporating the latest advancements and trends in diagnostics, therapies, and preventive medicine for civilization and age-related diseases into the education of students and medical graduates. With the combined research potential of the three faculties of the Jagiellonian University Medical College—Faculty of Pharmacy, Faculty of Medicine, and Faculty of Health Sciences—CDT-CARD ensures comprehensive coverage of key areas in pharmaceutical and medical sciences, ranging from fundamental research to treatment optimization and public health solutions.

The concept of the Centre is grounded in the belief that innovative therapeutic solutions can be developed through the following steps: (a) analysis of societal health needs, (b) research into the pathogenesis of civilization and age-related diseases using animal models that mimic these conditions, (c) identification of new therapeutic targets, (d) validation of novel therapeutic targets using in silico, in vitro, and in vivo models, (e) design of phase I clinical trials for selected candidate drugs, (f) prediction of disease progression and treatment efficacy using in silico models, and (g) identification of specific biomarkers for evaluating treatment effectiveness. This approach aims to enable the implementation of precision medicine principles in the treatment process.

The research infrastructure of CDT-CARD is based on core central laboratories (Core labs), ensuring a comprehensive and integrated approach to the design of new therapies. The Centre incorporates the following state-of-the-art laboratories equipped with cutting-edge instruments:

1. Genomics Laboratory
2. Proteomics Laboratory
3. Glycomics Laboratory
4. BioImaging Laboratory
5. Bioinformatics and In Silico Analysis Laboratory
6. Advanced Cell Culture Techniques and Microscopy Laboratory
7. Laboratory of Functional Analysis and Radioisotopes
8. Pharmacokinetics and Preliminary Toxicological Analysis Laboratory
9. Body Function Laboratory
10. Laboratory of Small Particles

In conclusion, the Centre for the Development of Therapies for Civilization and Age-Related Diseases (CDT-CARD) serves as a dynamic hub for interdisciplinary research, bringing together experts from various fields to tackle the challenges of civilization and age-related diseases. We invite researchers, scientists, and industry professionals to collaborate with us in advancing our understanding of these diseases and developing effective therapeutic interventions. Together, we can make significant strides in improving the health and well-being of individuals affected by civilization and age-related diseases.

We look forward to your participation and partnership.

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