21st International Congress
OF THE POLISH PHARMACOLOGICAL SOCIETY

Katowice, 28-30.09.2023

BOOK OF ABSTRACTS

POLSKIE TOWARZYSTWO FARMAKOLOGICZNE

Project entitled: ***21st International Congress of the Polish Pharmacological Society*** *co-financed from the State budget under the program of the Minister of Education and Science under the name „Excellent Science" project number: DNK/SN/550441/2022. Amount of financing PLN 121 089,00 total project value PLN 195 089,00.*

Projekt dofinansowany ze środków budżetu państwa w ramach programu Ministra Edukacji i Nauki pod nazwą XXI Międzynarodowy Zjazd Polskiego Towarzystwa Farmakologicznego *nr projektu* DNK/SN/550441/2022 *kwota dofinansowania 121089,00 PLN całkowita wartość projektu 195089,00 PLN.*

Table of contents

Nutritional ketosis and appetite in obese mice - behavioral and molecular studies

Mateusz Grabowski [1], Daniela Liśkiewicz [2], Konstancja Grabowska [1], Marta Nowacka-Chmielewska2 [2], Andrzej Małecki [2], Jarosław Barski [3]
[1]Department for Experimental Medicine, Medical University of Silesia in Katowice; Laboratory of Molecular Biology, The Jerzy Kukuczka Academy of Physical Education in Katowice, [2]Laboratory of Molecular Biology, The Jerzy Kukuczka Academy of Physical Education in Katowice, [3]Department for Experimental Medicine, Medical University of Silesia in Katowice

Obesity, a systemic disease with many comorbidities, has become a major public health problem. The first-line treatment for obesity is lifestyle intervention. However, over time, most nutritional plans prove to be ineffective for sustaining weight loss due to reduced motivation and increased appetite, resulting in gradual weight regain. In recent years low carbohydrate diets including the ketogenic diet (KD) are attracting increasing attention as a promising approach for weight loss. KD can be used as a treatment for many disorders, including obesity. Appetite suppression in the hypothalamus can be a crucial mechanism responsible for the efficiency of KD.
The aim of the study was to evaluate how the metabolic changes associated with obesity affect the mechanisms responsible for appetite regulation in nutritional ketosis.
10 weeks-old male mice were fed a high-caloric human snack-containing chow mimicking Western diet (diet-induced obesity, DIO model) to induce obesity for 6 weeks. Then, animals were divided into two groups and given either a KD (DIO-KD) or standard chow (DIO-SD) to reduce body mass. Meanwhile, the control mice received standard chow (CTL), and then were switched to KD (CTL-KD) or remained on standard chow (CTL-SD). QRT-PCR and ELISA were used to measure gene expression in the hypothalamus and serum concentration of selected factors associated with appetite regulation were measured. Moreover, nutritional behavior was performed to evaluate appetite by using the palatable meal test (PMT) and food-risk competition test (F/RCT). For statistical analysis, ANOVA tests were followed by Tukey's or Sidak's multiple comparison tests.
DIO and CTL mice fed with a KD showed lower body weight. Interestingly, caloric intake remained consistent regardless of whether the mice were fed a KD or SD. Notably, there were significant differences in serum beta-hydroxybutyrate, glucose, and insulin levels between groups. The obesity model had a significant impact on the serum concentration of ghrelin and leptin. KD altered the levels of *Agrp*, *Pomc*, and *Cart* genes, whereas the obesity model also affected the level of *Mc4r* gene expression in the hypothalamus. Additionally, there was a substantial increase in Pomc expression observed in the DIO-KD group when compared to the CTL-SD group. Animals in CTL-KD and DIO-KD groups ate less palatable snacks in the PMT and F/RCT tests in comparison to CTL-SD and DIO-SD groups.
Behavioral evaluation showed that the KD suppresses appetite in DIO and control mice. Calorie intake remained consistent during weight loss, regardless of diet type. Obesity affects the serum concentration of ghrelin and leptin, while the use of KD at the weight loss stage did not change their concentrations compared to the control group. In conclusion, nutritional ketosis affects the expression level of selected genes related to appetite regulation in obese and lean mice.

Impact of ovariectomy and metformin supplementation on the neuroinflammation-related changes in middle-aged mice.

Konstancja Grabowska [1], Mateusz Grabowski [1], Andrzej Małecki [2], Jarosław J. Barski [1], Marta Nowacka-Chmielewska [1]
[1]Department for Experimental Medicine, Faculty of Medical Sciences in Katowice, Medical University of Silesia, Katowice, Poland; Laboratory of Molecular Biology, Institute of Physiotherapy and Health Sciences,

Academy of Physical Education, Katowice, Polan, [2]Department for Experimental Medicine, Faculty of Medical Sciences in Katowice, Medical University of Silesia, Katowice, Poland

Neuroinflammation refers to an inflammatory response within the central nervous system. In postmenopausal women, estrogen deficiency may exacerbate central and peripheral inflammation with an increased predisposition to develop neuroinflammatory disorders. To prevent neuroinflammation pharmacological interventions like exercise mimetics (EMs), capable of activating cellular mechanisms similar to those observed after regular exercise – may be considered. Metformin (MF), a well-known anti-diabetic drug, was proposed as a one of the EMs. Recently, it was shown that MF may be a promising compound for reducing the proinflammatory response.
The study aimed to answer whether and how ovariectomy and MF supplementation influence neuroinflammation, including Nlrp3 inflammasome activation in the frontal cortices of middle-aged mice.
13-month-old female C57BL/6 mice underwent bilateral ovariectomy or sham operation. After a week of post-operation recovery, the mice were randomly assigned to four groups. MF was added to the drinking water at a dosage of 100 mg/kg/b.w./day for the next six weeks for both the ovariectomized (OVX/MF) and sham-operated (SHAM/MF) mice. Meanwhile, the control groups (SHAM/CL and OVX/CL) received drinking water without MF *ad libitum*. At the end of the experiment, frontal cortices and serum samples were collected for further evaluation. For all statistical analyses, two-way ANOVA followed by an appropriate post hoc test was performed.
Results show that the operation had an impact on the expression levels of the *Nlrp3* gene, while MF altered the expression of the *IL-18* gene. The interaction (operation x intervention) influenced the levels of Nlrp3 and pro-Il-18 proteins, whereas the intervention and operation changed the Asc protein level. The SHAM/MF and OVX/CL groups showed reduction in the Nlrp3 protein level when compared to SHAM/CL. The ovariectomy resulted in increase in the Asc level as compared to o SHAM/MF mice. However, no changes were observed in the gene expression levels of *Casp1* and *IL-1β*, nor their protein products pro-caspase1 and pro-Il-1β, respectively. Additionally, the interaction (operation x intervention) had an impact on the serum Il-18 concentration.
We concluded that ovariectomy and MF supplementation alter Nlrp3 inflammasome activation regulation in frontal cortices and serum of middle-aged females.
This research is supported by National Science Center grant no. 2021/41/N/NZ7/01581.

Key words: neuroinflammation, ovariectomy, metformin, exercise mimetics, Nlrp3 inflammasome

---

Amantadine - a new look at the old drug

Wojciech Danysz, Andrzej Dekundy
Merz Therapeutics, Eckenheimer Landstraße 100, D-60318, Frankfurt /M, Germany

Amantadine is a compound introduced into clinical practice over 50 years ago. Notwithstanding, new insights keep changing our look at this old drug and suggest its potential expansion of its therapeutic applications. Following on that, we attempted to re-appraise amantadine's mechanism of action by answering the two questions:
What concentrations of amantadine can be achieved at therapeutic doses, both extracellularly and intracellularly (the difference results from lysosomal accumulation)?
Which extracellular and intercellular targets can be affected by such concentration based on in vitro binding and/or functional data?
This analysis revealed that sigma-1 receptors, brain-derived neurotrophic factor (BDNF) and aromatic amino acids decarboxylase (AADC) are the targets which are more likely than the generally favoured NMDA receptor. Interestingly enough, all these targets acting in concert may contribute to amantadine's therapeutic effects in Parkinson's disease, levodopa-induced dyskinesia and, potentially, neuroprotection.
The aforementioned targets have been implicated in the traumatic brain injury (TBI) and there is at least preclinical evidence that their modulation may enhance recovery from TBI and potentially provide neuroprotection.

In fact, there are also numerous clinical data showing benefit of amantadine treatment in TBI ranging from acceleration of awakening to better recovery of cognitive and motor functions. However, a randomized controlled clinical trial providing a clear-cut proof that these effects are due to neuroprotection and not solely to functional enhancement is still lacking.
Last but not least, we would like to emphasize that in spite of clinical publications suggesting benefit from treatment of COVID-19 patients, there is currently no evidence that such an effect exists. Based on the targets affected by amantadine at therapeutic doses there is no indication that such an activity can be expected.

Effect of resveratrol on kynurenic acid production in rat brain: study in vivo.

Katarzyna Wicha-Komsta , Tomasz Kocki
[1]Department of Experimental and Clinical Pharmacology, Faculty of Medicine, Medical University of Lublin, Jaczewskiego 8b, 20-090 Lublin, Poland, Lublin, Poland, [2]Department of Experimental and Clinical Pharmacology, Faculty of Medicine, Medical University of Lublin, Jaczewskiego 8b, 20-090 Lublin, Poland, Lublin, Poland

Kocki T [1] , Wicha-Komsta K [1]
[1] Department of Experimental and Clinical Pharmacology, Faculty of Medicine, Medical University of Lublin, Jaczewskiego 8b, 20-090 Lublin, Poland, Lublin, Poland

Introduction Resveratrol (RSV) is natural poliphenolic phytoalexine widely occuring in plants from Vaccinium sp (cranberries, blueberies, redberries), Vitis sp. (Vitis vinifera L., V. amurensis, V. cinerea (GH)). A lot of studies showed that resveratrol displays potential pharmacology effects including modulating immune response, antioxidant, anticancer, neuroprotective and anti-inflamation properties. Kynurenic acid (KYNA) is an endogenous brain constituent that inhibits the activity of all three ionotropic excitatory amino acid (EAA) receptors. KYNA is capable of preventing the neurodegeneration induced by experimental application of EAA receptor agonists. The possible role of altered KYNA-mediated modulation of EAA receptors in the human neuropathology has been postulated. In particular the disturbances of KYNA production have been linked to the occurrence of epilepsy, Huntington's disease, Alzheimer's disease, schizophrenia, AIDS-related dementia and others.
Aim of the study
The aim of this study was to evaluate the effect of RSV on KYNA synthesis in rat brain cortex, hippocampus and striatum. Material and methods
The research was carried out on Male Wistar rats. After 14- days of daily intraperitoneal administration of RSV (5, 10, 20, 40 mg/kg of body weight; n=6) the rats was decapitated. Brain cortex, striatum and hippocampus was separated and the amount of KYNA was determined by HPLC method with fluorescence detection. The prepared samples were separated on analytical column (Agilent HC-C18 (2); 250× 4.6 mm i.d.; 5 m particle size). The mobile phase was composed of 20 mmol/L NaAc, 3 mmol/L ZnAc2 and 7% acetonitrile. KYNA was quantified fluorometrically (excitation 344 nm with detection at emission 398 nm). Statistical significance was determined using the Kruskal-Wallis non-parametric test due to non-normally distributed data. Results RSV at the concentration of 10; 20; 40 mg/kg significantly decreased KYNA production in the brain cortex ($P<0.05$ vs control) while RSV at the dose of 10; 20; 40 mg/kg or 20; 40 mg/kg increased synthesis of KYNA in striatum ($P<0.05$ vs control) and in hippocampus ($P<0.05$ vs control), respectively. Conclusion. The data obtained here suggest that RSV might change KYNA production in rat brain.
Acknowledgements
Supported by the grant from Medical University of Lublin, No DS 457

Does the blood-brain barrier play a role in the stress response?

J. Solich , M. Dziedzicka-Wasylewska
Maj Institute of Pharmacology Polish Academy of Sciences, Kraków, Poland

Introduction: Stress is a the biological response induced by the intrinsic or extrinsic stimulus with consequences for the body. However, the individual response to stress might be different: its either susceptibility or resilience to the adverse conditions, what can be modelled on animals. This approach led us to study three mouse strains demonstrating various stress-coping strategies: C57BL/6J, norepinephrine transporter knock-out mice and SWR/J mice. 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. Further analyses indicated that transcripts targeted by these miRNAs encode proteins responsible for blood brain barrier (BBB) development and function.
Aim of the study: The aim of the current study was to determine the levels of transcripts targeted by miRNAs mentioned above and their protein products in the brains of three strains of mice subjected to restraint stress.
Material and methods: To achieve this goal RT-qPCR reactions and immunohistochemistry were used to determine mRNAs encoding BBB-associated proteins, and the distribution of proteins, in four different brain regions of mice.
Results: The most interesting results concern stress-induced down-regulation of mRNA encoding claudin-5 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. The highest alterations in mRNAs differentiating stress-resilient from stress-susceptible mice were detected in the hippocampus, less – in the prefrontal cortex and cerebellum, and the least - in the nucleus accumbens. Changes in the levels of some BBB-related proteins confirmed the differences between stress-resilient and stress-susceptible mice.
Conclusions: The differences in mRNAs expression and protein levels could be involved in the differential permeability of the BBB of stress-resilient as compared to stress-susceptible animals. 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.
Acknowledgements: 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.

Abstract topics:

• Blood-brain barrier pharmacology

Key words: blood-brain

Glial Fibrillary Acidic Protein as a marker of dexamethasone-induced neurotoxicity? The preliminary study.

Danilczuk Zofia [1], Krasuska-Grzegorczyk Beata [2], Dobrowolski Piotr [3]
[1]Department of Experimental and Clinical Pharmacology, Medical University of Lublin, Jaczewskiego 8b, 20-090 Lublin, Poland, [2]Chair and Department of Cardiology, Medical University of Lublin, Jaczewskiego 8, 20-954 Lublin, Poland, [3]Department Functional Anatomy and Cytobiology Maria Curie-Sklodowska University, Lublin, Poland

GFAP is a marker for astrocytes, known to be induced upon brain damage or during CNS degeneration and to be more highly expressed in the aged brain. Many studies have revealed the functions of GFAP in astrocytes,

such as migration, functioning of the blood barier, signal transduction pathways and neuron-glia interactions. It turned out that many types of CNS injury, eg. cerebral ischemia by inducing a significant depression in hippocampal slices devoid of GFAP, caused a loss of pyramidal neurons in mice. It has been suggested that neuronal death is increased when GFAP is absent in injury conditions. Recent study has demonstrated that chronic exposure to elevated levels of glucocorticoids (GCs) results in neurodegenerative changes in the central nervous system, particularly in the limbic system and hippocampus, structures regulating behaviour, memory and other cognitive functions. The aim of the immunohistochemical study was to assess the impact of dexamethasone (DEX- a synthetic agonist of GCs) on GFAP in the CA3 subfield of hippocampus in mice. The study was performed in the animal model of neurotoxicity induced by administration of DEX to Albino mice at the dose of 16 mg/kg/day for 14 days. 48 h after the last injection of drug mice were anesthetised and the brains of mice were subjected to immunohistochemical examination.The immunohistochemical staining using Monoclonal Mouse Anti-human Gial Fibrillary Acidic Protein was performed. The ratio of immunopositive cells to the total number of cells (average %) in area of hippocampal CA3 subfield was determined. What was also assessed was average intensity of immunoreaction [8 bit gray scale] on GFAP, in pyramidal layer of hippocampal CA3 subfield. The results of the experiments are expressed as the mean ± SEM. The data were assessed by one-way analysis of variance (ANOVA) and Tukey-Kramer post test. The analysis showed significant differences in the content of GFAP-positive cells on the hippocampal cross-section and in the intensity of the immunohistochemical reaction in the DEX group compared to control mice, which can be interpreted as a semi-quantitative increase in the amount of labelled protein. The results of these studies suggest a protective involvement of GFAP in the damage to hippocampal pyramidal neurons caused by chronic dexamethasone administration. Elucidation of this effect requires further study.

Key words: dexamethasone, neurotoxicity, GFAP

Reduction of mitochondrial Activity by Tetrahydrocannabinol and Cannabidiol: Curse or Blessing?

Rudolf Moldzio [1], Marcin Delijewski [2], Christopher Krewenka [1]
[1]*University of Veterinary Medicine, Vienna, Austria,* [2]*Medical University of Silesia, Katovice, Poland*

Introduction:
Tetrahydrocannabinol (THC) and other phytocannabinoids are considered to have beneficial and neuroprotective effects on a variety of neuronal diseases, while cannabidiol (CBD) in addition is discussed as a potential drug in cancer therapy. Many of these effects have been reported by physicians on *Cannabis* consuming patients or by users in an anecdotal manner. With the legalization of THC in many countries and the current widespread use of CBD, a more complete understanding of these drug´s mode of action is needed. In our study, we aimed to know how THC and CBD act on neuronal mitochondria, and whether these effects might explain the neuroprotection we found in a cell culture model for Morbus Parkinson (PD).
Material and Methods:
We examined the effects of THC and CBD on enzymes of the respiratory chain, while neuroblastoma cells and primary mesencephalic cells were used to measure oxygen consumption and extracellular acidification rates. In addition, we studied phytocannabinoids in these cells co-administered with complex I inhibitor rotenone, a well-described model compound for PD. Data were statistically evaluated by the Kruskall/Wallis followed by a Chi^2 test.
Results:
THC and CBD reduce enzymes of the respiratory chain and ATP formation to a similar extend, while oxygen consumption was more reduced in CBD-treated cell cultures. In mesencephalic cultures, each cannabinoid

reduces also glycolysis. The reduction was more pronounced in CBD-treated cultures, which corresponds to the finding that CBD, but not THC is toxic to neurons in culture. CBD is usually considered to have no degenerative potential, which is not supported by our study. Nonetheless, data reveal that both cannabinoids counteract rotenone-induced degeneration significantly. The underlying mechanisms are still unknown, but are shown to be cannabinoid receptor 1 independent.
Conclusion:
To summarize, we hypothesize that cannabinoids put neurons into a “quiescent state” that helps them to withstand periods of mitochondrial stress. Our cell culture data are in accordance to reports about the beneficial effect of THC on PD patients, while we are not convinced of the harmlessness of CBD.

The study was supported by the Austrian Research Promotion Agency, the Herzfelder Family Foundation and the Bionorica Research GmbH.

Key words: Phytocannabinoids, Mitochondria, Morbus Parkinson

Physiological effects of a two-fold dietary copper and zinc administration in aged rats

Michał Majewski
Department of Pharmacology and Toxicology, Faculty of Medicine, University of Warmia and Mazury, Olsztyn, Poland

Dietary copper (Cu) and zinc (Zn) fluctuations play a key role in the development of cardiovascular disorders. The aim of this study was to analyze the impact of increased dietary Cu and Zn on the cardiovascular functioning. Male Wistar rats at 12 months of age were fed for 8 weeks with either recommended (6.5 and 15.9 mg/kg) or enhanced (13.0 and 31.8 mg/kg) levels of Cu and Zn, respectively. Blood, hearts and aortic rings were taken for further analysis. Two-fold increase in Cu and Zn increased TAS (by 1.6-fold) and decreased Cu (0.6-fold) and Cu/Zn ratio (0.6-fold) in blood plasma (ASA). Neither Zn, Fe, Se nor HO-1, COX-1, COX-2, NOS3, GAPDH, ICAM-1 (ELISA) were modified. Vasodilator response to acetylcholine of isolated aortic rings precontracted with noradrenaline was neither changed under the control condition nor after the pre-incubation with NS-398, TCP, AH6809, AL8810. Isolated heart functioning was not modified. Neither the weight of internal organs nor the body weight/fat composition were changed. Prolonged administration of a two-fold Cu and Zn, increased the antioxidant status of blood plasma and decreased the Cu content in blood plasma, however had no effect on functioning of cardiovascular system.

Key words: Cu, Zn, thoracic arteries, heart, antioxidant status

---

Shaken, not stirred – in search for new potential pharmacotherapy of essential tremor

Barbara Kosmowska
Maj Institute of Pharmacology Polish Academy of Sciences

Essential tremor (ET) is one of the most common movement disorders manifested by severe tremor in the upper limbs, negatively affecting patients' quality of life. Since its pathomechanisms are still unclear, there are also no approved tremor-selective pharmacotherapies and only drugs developed for other disease entities, such as propranolol, are used in the treatment of ET. Unfortunately, in more than 30% of patients such pharmacotherapy is ineffective. Therefore, there is an urgent need to look for effective and safe tremor-focused therapies.

One of the most recognized animal models of ET is acute harmaline-induced tremor, generating 10-12 Hz whole-body tremor in rats. Harmaline acting on the inferior olive nuclei (ION) leads to abnormal synchronous activation of glutamatergic climbing fibers that form synapses on the Purkinje cells of the cerebellar cortex, causing characteristic discharges and increased glutamate (GLU) release in the cerebellar cortex and thalamic motor nuclei, as well as in the cerebral cortex.
To search for a new ET therapy, several different compounds were tested for antitremor properties in the harmaline model, such as ligands of adenosine A1, dopamine D2/D3 or $GABA_A$ receptors. All of them showed anti-tremor potential. Both, a selective A1 adenosine receptor agonist (5'Cl-ENBA) as well as D3/D2 receptor agonist (pramipexole) reversed activation of the ION, motor thalamus, and motor cortex (measured by zif-268 mRNA expression). Furthermore, the A1 receptor agonist blocked harmaline-induced increased GLU release in the motor nuclei of the thalamus and harmaline-evoked apoptosis in the same structure. Positive allosteric modulators of the $GABA_A$ receptor, selective for the α2/3 subunit, but not α1, show an interesting behavioral anti-tremor profile of changes.
The obtained results indicate that both A1 and $GABA_A$α2/3 receptor ligands may be a potential target in ET therapy. It also seems that restoring homeostasis between excitatory and inhibitory transmission in the olivo-cerebello-thalamo-cortical pathway may be the key to developing a new effective ET therapy.

Key words: essential tremor, harmaline model, adenosine, glutamate, GABA

The effect of arylpiperazine derivatives on behaviour of larval zebrafish

Natalia Karkoszka [1], Kinga Gawel [2], Nataliia Kosheva [2], Ewa Kedzierska [3]
[1]Department of Pharmacology with Pharmacodynamics, Medical University of Lublin, Chodźki Str. 4a, 20-093 Lublin/ Department of Experimental and Clinical Pharmacology, Medical University of Lublin, Jaczewskiego Str. 8b, 20-090 Lublin, Poland, [2]Department of Experimental and Clinical Pharmacology, Medical University of Lublin, Jaczewskiego Str. 8b, 20-090 Lublin, Poland, [3]Department of Pharmacology with Pharmacodynamics, Medical University of Lublin, Chodźki Str. 4a, 20-093 Lublin

Many studies point that substances acting on serotonergic transmission are a target in the search for new, more effective psychotropic drugs used in the treatment of central nervous system diseases. These substances appear to have a better safety profile because they modulate rather than directly mediate neurotransmission in areas of the brain involved in the development of depressive and anxiety disorders: they do not cause sedation, memory impairment, do not interact with alcohol, and have no addictive potential. Thus, the aim of this work was to examine potential central activity of the seven new compounds from the group of arylpiperazine derivatives with the affinity to serotonin 5-HT_{1A} and/or 5-HT_{2C} receptors.
The compounds were studied to determine their effect on the behaviour of larval zebrafish (*Danio rerio*). To do so, 4 days post-fertilization (dpf) zebrafish were incubated for 24 h in maximal tolerated concentrations of solutions of different derivatives and subsequently submitted to behavioural analysis. Basic locomotor activity and light-dark transition (anxiety) assays were performed using Noldus apparatus (Netherlands). One way ANOVA with Tukey's *post-hoc* showed that among 7 tested compounds, derivative nr 1, 8 and 18 decreased larval basic locomotor activity. Similarly, those three compounds did affect behaviour of zebrafish in light-dark transition assay, decreasing larval movements compare to control counterparts in the dark phase of assay.
In conclusion, it seems that selected arylpiperazine derivatives affect central nervous system function but further study is needed to determine whether they might be used as anxiolytics.
Acknowledgment: The study was partially supported by DS 19, Medical University of Lublin (EK) and DS 448, Medical University of Lublin (KG).

Key words: arylpiperazine derivatives; zebrafish behaviour; anxiety

---

Urolithin A: gut-initiated neuroprotection in the battle against Parkinson's disease

Małgorzata Kujawska
Department of Toxicology, Poznan University of Medical Sciences, Dojazd 30, 60-631 Poznań, Poland

Parkinson's disease (PD) is a movement neurodegenerative disorder with prevalence increasing with age. The hallmarks of PD are misfolding and aggregation of a-synuclein, abnormal proteostasis, impaired energy homeostasis, gene mutations, neuroinflammation, and neuronal loss. PD patients usually receive a diagnosis when they develop motor symptoms, but there is already a significant loss of dopaminergic neurons, accumulation of a-synuclein assemblies in the surviving neurons, and neuroinflammation. Disease management includes symptom alleviation; however, no disease-modifying options are available.
Given the multifactorial neurodegenerative process in PD, combinatorial and multi-targeted therapies to halt the disorder are considered. In this context, the holistic neuroprotection strategy to protect neurons from degeneration is gaining attention. Therefore, identifying neuroprotective compounds and nutritional interventions is a research hotspot. Polyphenols and their metabolites exerting various biological activities have been highlighted as a valuable source for strengthening neuroprotection. Many studies have shown the health benefits of urolithin A (UA) – a metabolite formed by the gut microbiota following the consumption of dietary ellagitannins. A growing literature explores the role of this metabolite in mitochondrial health and its anti-inflammatory, antioxidant, anti-apoptotic and autophagy enhancer properties, which are critical in the battle against PD. Importantly, UA is distributed to the brain. However, only a few studies support its relevance to PD. In rodent models of PD, UA administration protected against motor deficits accompanied by a decreased loss of dopaminergic neurons and ameliorated neuroinflammation. The mechanistic study revealed favourable involvement of mitophagy, improvement in mitochondrial functions, enhancement of protein clearance and inhibition of the pro-inflammatory response upon UA treatment. Moreover, UA can exert a neuroprotective effect through the gut-brain axis as it can modulate intestinal integrity and the colonic immune milieu. Increased anti-inflammatory colonic γδ T cells responsible for intestinal repair in aged PD mice upon dietary intervention with UA were associated with improved cognitive behaviour.
Accordingly, advanced nutritional approaches enabling the delivery of UA in a calibrated manner are considered to manage the natural heterogeneity of the gut microbiome for holistic neuroprotection.

Key words: gut microbiota, metabolite, neuroprotective, mitophagy, anti-inflammatory

---

Modulating effect of Scutellaria baicalensis and its flavonoids on the hypotensive effect of methyldopa in pregnant SHR rats

Michał Szulc
Department of Pharmacology, Poznań University of Medical Sciences

The study aimed to investigate the interaction of *Scutellaria baicalensis* root extract and its flavonoids baicalein and chrysin with methyldopa in pregnant, spontaneously hypertensive rats (SHR) at the pharmacodynamic, molecular, and biochemical levels. The rats, after confirming pregnancy, received *Scuttelaria baicalensis* extract (1.000 mg/kg/day, p.o.), baicalein (200 mg/kg/day, p.o.) and chrysin (200 mg/kg/day, p.o.) in combination with methyldopa (400 mg/kg/day; p.o.), for 14 consecutive days, twice daily, 1 h before morning and evening blood pressure and heart rate measurements (measured 3 times daily). Root extract from Scutellaria baicalensis, baicalein, and chrysin co-administered with methyldopa was associated with reduced blood pressure, especially during the first three days. In mothers' hearts and placenta after giving birth to their offspring, mRNA expression of factors related to inflammatory processes and vascular diseases was measured. Levels of oxidative stress markers in the placenta and indicators of myocardial damage in the heart were also assessed. The interactions were more pronounced for such factors as TGF-β, HIF- 1α, VEGF, and PlGF than TNF-α, Il-1β, and IL-6. Combined application of selected flavonoids and extract with methyldopa may be of

value in developing a new antihypertensive medication for patients suffering from preeclampsia or pregnancy-induced hypertension.

Key words: Scutellaria baicalensis; baicalein; chrysin, methyldopa; hypertension; blood pressure; mRNA expression; inflammatory and vascular factors; oxidative stress

---

Analgesic and antiinflammatory activity of Chelidonium majus alcaloids.

Beata Nowak, Maciej Danielewski, Sylwia Zielińska, Maciej Włodarczyk, Anna Jezierska-Domaradzka, Wojciech Słupski, Anna Merwid-Ląd, Marta Sznadruk-Bender, Piotr Ziółkowski, Adam Szeląg
Wroclaw Medical University

Introduction: Pain treatment is an challenge for contemporary medicine. On one hand nonsteroidal anti-inflammatory drugs are not deprived of side effects, of which ulcerogenic activity is one of the most frequent. On the other hands, opoids that do not exert negative influence on gastrointestinal tract have no anti-inflammatory potency and their usage is associated with high risk of addiction. The aims of the presented studies were to evaluate the analgesic and anti-inflammatory activity of Chelidonium majus alkaloids: sanguinarine and chelerithrine, and their influence on the integrity of gastric mucosa.
Material and methods: All experimens were conducted on male Wistar rats. The first experiment assessed the influence of sanguinarine and chelerithrine mixture (SC) (1:5) (1, 5, 10 mg/kg i.g.); on the carrageenan-induced paw oedema and PGE2 release, TNFα production, and MMP-9 concentration in inflamed tissue were determined. Additionally, the macroscopic and microscopic damage of gastric mucosa was evaluated. In the second experiment analgesic effect of SC (1:5) (5, 10 mg/kg) as well as sanguinarine (SANG) and chelerithrine (CHEL) in doses responding the doses in the mixture was assessed in tail-flic test. The last experiment we investigated the analgesic and antinflamamtory activity of sanguinarine and chelerithrine mixture (1:5) (5, 10 mg/kg) as well as sanguinarine and chelerithrine in doses responding the doses in the mixture in formalin test.
Results: Administration of SC dose-dependently inhibited the second phase of carrageenan rat paw oedema and PGE2 release, decreased the production of TNFα, and reduced the concentration of MMP-9, and the efficacy of the highest dose was comparable to the effect of IND. Contrary to IND, no gastrotoxic activity of SC was detected. SANG, CHEL and SC significantly prolong the time of the latency in tail-flick test. However only the highest doe of CHEL exert analgesic effect comparable to morphine, suggesting it central analgesic activity. In formalin test only highest doses of SANG, CHEL and SC reduced significantly the time of licking. However, only SC and SANG additionally reduced the formalin-induced synthesis of TNFα and MMP-9.
Conclusions: The investigated sanguinarine: chelerythrine mixture seems to be a promising candidate for further research on new anti-inflammatory and analgesic drugs characterized with a safer gastric profile compared to existing NSAIDs.

Key words: inflammation; pain; sanguinarine, chelerithrine

Aspalathus linearis infusion affects social behaviour of Sprague-Dawley rats and modifies activity of hypothalamic BDNF/TrkB pathway.

Justyna Pyrzanowska , Ilona Joniec-Maciejak , Adriana Wawer , Ewa Machaj , Kamilla Blecharz-Klin , Agnieszka Piechal , Dagmara Mirowska-Guzel
[1]Department of Experimental and Clinical Pharmacology, Medical University of Warsaw, CePT, Banacha 1b, 02-097 Warszawa, [2]Department of Experimental and Clinical Pharmacology, Medical University of Warsaw, CePT, Banacha 1b, 02-097 Warszawa, [3]Department of Experimental and Clinical Pharmacology, Medical University of Warsaw, CePT, Banacha 1b, 02-097 Warszawa, [4]Department of Experimental and Clinical

Pharmacology, Medical University of Warsaw, CePT, Banacha 1b, 02-097 Warszawa, [5]Department of Experimental and Clinical Pharmacology, Medical University of Warsaw, CePT, Banacha 1b, 02-097 Warszawa, [6]Department of Experimental and Clinical Pharmacology, Medical University of Warsaw, CePT, Banacha 1b, 02-097 Warszawa, [7]Department of Experimental and Clinical Pharmacology, Medical University of Warsaw, CePT, Banacha 1b, 02-097 Warszawa

Rooibos herbal tea, made of *Aspalathus linearis* (Brum.f) Dahlg. (*Fabaceae)* leaves, exhibits cell protective activity and ability to attenuate metabolic syndrome/cardiovascular risk as well as to support neuroprotection. Rooibos tea modifies neurotransmission in several brain structures as well as improves memory and increases exploratory activity of rats. Nevertheless little is known about the impact of rooibos tea on hypothalamus that controls neuroendocrine, autonomic and environmental inputs and contributes to behavioural expression of emotions including inter alia social interactions.
The experiment evaluated the effects of 3 month oral administration of "fermented" rooibos herbal tea (FRHT) to adult male SD rats on behaviour as well as on neurotrophin pathway BDNF/TrkB activity and neurotransmission in the hypothalamus. Infusions (1, 2 and 4 g of leaves for 100 ml of boiling water) were chromatographically tested for flavonoid content. The behaviour of animals was examined in social interaction test. Brain-derived Neurotrophic Factor (BDNF) and Tropomycin Receptor Kinase B (TrkB) content in whole the hypothalamus were yielded by ELISA kits, whereas monoamines, their metabolites as well as amino acids by HPLC-ECD. Statistical analysis used Kruskal–Wallis ANOVA, Dunn's multiple comparison test and Mann–Whitney test (MW).
The results revealed that FRHT administration significantly decreased total time spent on social contact during the trial in all animal groups ($H_{(3,17)}$=8.14, p=0.04; p<0.05 vs Con, MW) and total time of active social interactions ($H_{(3,17)}$=8.28, p=0.04; p<0.05 vs Con, MW). No aggressive behaviour was observed between the rats. The level of BDNF in whole the hypothalamic structure remained unchanged in all the groups of animals ($H_{(3,35)}$=0.93, p=0.82), whereas the TrkB concentration was smaller in all rooibos-treated rats ($H_{(3,35)}$=13.98, p=0.003; p<0.01 vs Con, MW). Also a decreased level of serotonin in animals treated with the highest concentration of the infusion ($H_{(3,35)}$=13.83, p=0.003; p<0.01 vs Con, MW) was seen. The 2:100 infusion increased level of glutamic acid ($H_{(3,35)}$=12.99, p=0.005; p<0.01 vs Con, MW).
In conclusion rooibos tea consumption was able to affect social behaviour of rats and decrease the activity of BDNF/TrkB pathway in whole the hypothalamus. The results suggest possible influence of rooibos tea on hypothalamic functions that need further detailed investigation focusing on particular hypothalamic nuclei.

Key words: Aspalathus linearis, Rooibos tea, Hypothalamus, Social behavior, BDNF/TrkB

The effect of valproate on the amino acids, monoamines and kynurenic acid level in the brain structures of the pentylenetetrazol kindled rats

Aleksandra Wisłowska-Stanek , Danuta Turzyńska [2], Karolina Kołosowska [2], Anna Skórzewska [2], Alicja Sobolewska [2], Janusz Szyndler , Piotr Maciejak [4]
[1]Department of Experimental and Clinical Pharmacology, Medical University of Warsaw, Poland;, [2]Department of Neurochemistry, Institute of Psychiatry and Neurology, 9 Sobieskiego Street, 02-957 Warsaw, Poland, [3]Department of Experimental and Clinical Pharmacology, Medical University of Warsaw, Poland;, [4]1Department of Experimental and Clinical Pharmacology, Medical University of Warsaw, Poland; Centre For Preclinical Research and Technology (CePT), 1B Banacha Street, 02-097 Warsaw, Poland; Department of Neurochemistry, Institute of Psychiatry and Neurolo

Introduction: About 30% of epileptic patients do not respond to the first-line anticonvulsants. PTZ-induced kindling is a well-established model of temporal lobe epilepsy – most common form of focal epilepsy. Valproate has been proved to be efficient and safe in treatment of many types of epilepsy, e.g. in generalized (absence, tonic-clonic, myoclonic) and partial (simple, complex, secondarily generalized) seizures, so analyzing its molecular mechanism of action seems very useful to look for new more effective drugs.

Aim of the study: The aim of the study was to assess the influence of a single valproate (VPA) administration on the amino acids, monoamines and kynurenic pathway metabolites' levels in brain structures involved in epileptogenesis (hippocampus, amygdala, prefrontal cortex, and striatum) in pentylenetetrazol (PTZ)-kindled rats.
Materials and methods: Adult Wistar rats were used in this study. Kindled rats received repetitive injection of PTZ at a subconvulsive dose, three times per week. Animals were considered as kindled after they exhibited stage 4 or 5 according to the Racine scale in two consecutive trials. On the experimental day, VPA group received acute single dose of VPA 400 mg/kg intraperitoneally, saline group received 0.9% NaCl. After 30 minutes, both groups obtained PTZ 30 mg/kg. The concentration of amino acids, kynurenic acid, monoamines were assessed in the cortex, hippocampus, striatum, amygdala by high performance liquid chromatography. The differences in neurotransmitters between kindling rats after saline or valproate administration were analyzed by Student-t test.
Results: It was found that VPA increased the gamma-aminobutyric acid (GABA), tryptophan, 5-hydroxyindoleacetic acid, kynurenic acid (KYNA) concentrations and decreased aspartate levels in all analyzed structures. Moreover, VPA heightened the level of 3,4 dihydroxyphenylacetic acid and homovalinic acid in the prefrontal cortex and striatum.
Conclusions: Our results indicate that a single administration of VPA in the PTZ-kindled rats restored proper balance between excitatory and inhibitory neurotransmition by decreasing the level of aspartate and increasing the levels of GABA and KYNA and affecting serotoninergic and dopaminergic neurotransmission in the prefrontal cortex, limbic structures and striatum.
Acknowledgements: The work was supported by Grant 2018/28/C/NZ7/00240 from the National Science Centre in Poland.

Key words: kindling, valproate, amino acids, kynurenic acid, monoamines, hippocampus, amygdala, prefrontal, cortex, striatum

---

The effect of valproate on the amino acids, monoamines and kynurenic acid level in the brain structures of the pentylenetetrazol kindled rats

Aleksandra Wisłowska-Stanek , Danuta Turzyńska [2], Karolina Kołosowska [2], Anna Skórzewska [2], Alicja Sobolewska [2], Janusz Szyndler , Piotr Maciejak [4]
[1]Department of Experimental and Clinical Pharmacology, Medical University of Warsaw, Poland;, [2]Department of Neurochemistry, Institute of Psychiatry and Neurology, 9 Sobieskiego Street, 02-957 Warsaw, Poland, [3]Department of Experimental and Clinical Pharmacology, Medical University of Warsaw, Poland;, [4]1Department of Experimental and Clinical Pharmacology, Medical University of Warsaw, Poland; Centre For Preclinical Research and Technology (CePT), 1B Banacha Street, 02-097 Warsaw, Poland; Department of Neurochemistry, Institute of Psychiatry and Neurolo

Introduction: About 30% of epileptic patients do not respond to the first-line anticonvulsants. PTZ-induced kindling is a well-established model of temporal lobe epilepsy – most common form of focal epilepsy. Valproate has been proved to be efficient and safe in treatment of many types of epilepsy, e.g. in generalized (absence, tonic-clonic, myoclonic) and partial (simple, complex, secondarily generalized) seizures, so analyzing its molecular mechanism of action seems very useful to look for new more effective drugs.
Aim of the study: The aim of the study was to assess the influence of a single valproate (VPA) administration on the amino acids, monoamines and kynurenic pathway metabolites' levels in brain structures involved in epileptogenesis (hippocampus, amygdala, prefrontal cortex, and striatum) in pentylenetetrazol (PTZ)-kindled rats.
Materials and methods: Adult Wistar rats were used in this study. Kindled rats received repetitive injection of PTZ at a subconvulsive dose, three times per week. Animals were considered as kindled after they exhibited

stage 4 or 5 according to the Racine scale in two consecutive trials. On the experimental day, VPA group received acute single dose of VPA 400 mg/kg intraperitoneally, saline group received 0.9% NaCl. After 30 minutes, both groups obtained PTZ 30 mg/kg. The concentration of amino acids, kynurenic acid, monoamines were assessed in the cortex, hippocampus, striatum, amygdala by high performance liquid chromatography. The differences in neurotransmitters between kindling rats after saline or valproate administration were analyzed by Student-t test.
Results: It was found that VPA increased the gamma-aminobutyric acid (GABA), tryptophan, 5-hydroxyindoleacetic acid, kynurenic acid (KYNA) concentrations and decreased aspartate levels in all analyzed structures. Moreover, VPA heightened the level of 3,4 dihydroxyphenylacetic acid and homovalinic acid in the prefrontal cortex and striatum.
Conclusions: Our results indicate that a single administration of VPA in the PTZ-kindled rats restored proper balance between excitatory and inhibitory neurotransmition by decreasing the level of aspartate and increasing the levels of GABA and KYNA and affecting serotoninergic and dopaminergic neurotransmission in the prefrontal cortex, limbic structures and striatum.
Acknowledgements: The work was supported by Grant 2018/28/C/NZ7/00240 from the National Science Centre in Poland.

Key words: kindling, valproate, amino acids, kynurenic acid, monoamines, hippocampus, amygdala, prefrontal, cortex, striatum

---

Anticancer activity of 7-methoxy-2-phenylbenzo[b]furan-3-yl)(3,4,5-trimethoxyphenyl)methanone against primary and metastatic melanoma cells

Magdalena Perużyńska [1], Radosław Birger [1], Katarzyna Piotrowska [2], Halina Kwiecień [3], Marek Droździk [1], Mateusz Kurzawski [4]
[1]Department of Experimental & Clinical Pharmacology, Pomeranian Medical University in Szczecin, Powstancow Wlkp. 72, 70–111 Szczecin, Poland, [2]Department of Physiology, Pomeranian Medical University in Szczecin, Powstancow Wlkp. 72, 70–111 Szczecin, Poland, [3]Faculty of Chemical Technology and Engineering, West Pomeranian University of Technology in Szczecin, Piastów Ave. 42, 71-065 Szczecin, Poland, [4]Laboratory of Pharmacodynamics, Pomeranian Medical University in Szczecin, 71-899 Szczecin, Poland

Cutaneous melanoma is the most common form of malignant tumor, derived from melanocytes. Moreover, it remains the most lethal form of skin cancer, as the success of the therapy strictly depends on the stage of the disease at the time of diagnosis. Herein, we report the results of evaluation of anticancer activity of 7-methoxy-2-phenylbenzo[b]furan-3-yl)(3,4,5-trimethoxyphenyl)methanone (BF3) against primary (A375) and metastatic (MDA-MB-435S) melanoma cells.
First, anticancer potential of BF3 against a wide panel of 60 different human tumor cell lines was evaluated under the drug discovery program of the National Cancer Institute. Next, antiproliferative activity of the compound was assessed using formazan-based assay against selected melanoma cell lines. To determine the mode of action of BF3 its effect on intracellular microtubule networks, cell cycle phase distribution, and apoptosis was evaluated. Furthermore, molecular modeling studies of the colchicine binding site of tubulin were performed. Differences between the BF3-treated and control (non-treated) cells, as well as differences between primary tumor and metastatic cell lines, were assessed by t-student test.
The NCI-60 screening results highlighted the anticancer potential of BF3 against different cancer cell types, especially colon cancer, central nervous system (CNS) cancer and melanoma. Our study revealed that BF3 inhibited cell proliferation in a dose dependent manner with IC_{50} values yielding 0.09±0.01 μM and 0.11±0.01 μM against A375 and MDA-MB-435S cells, respectively. Further study, showed that the strong antiproliferative activity of compound BF3 correlated well with its inhibitory effect on tubulin polymerization

(with IC_{50} values equal: 0.55±0.06 μM and 0.82±0.27 μM for A375 and MDA-MB-435S cells, respectively). The obtained molecular docking results allow to claim that BF3 belongs to the colchicine binding site inhibitors (CBSIs), and similarly to prototype colchicine displayed hydrogen-bonding interactions with the sulfur atom of βCys241. As expected, similarly to other tubulin destabilizing agents, BF3 disturbed cell cycle progression, leading to G2/M arrest and apoptosis.
Our study confirmed promising anticancer activity of BF3 and showed that melanoma cell line that originated from primary neoplasm was more sensitive to tested compound than cell line derived from a metastatic site.
Acknowledgements: We are grateful to the National Cancer Institute for anticancer screening study.

Key words: melanoma, benzo[b]furans, anticancer agents, antitubulin agents

---

Hyperbaric Oxygen Therapy (HBOT) – as multilevel adjuvant therapy targeting in sensitizing glioma cells to therapy .

Anna Bielecka-Wajdman [1], Grzegorz Machnik [1], Tomasz Cichoń [2], Natalia Hajduga [1], Katarzyna Jaskiewicz [1], Ewa Obuchowicz [1]
[1]Medical University of Silesia in Katowice, [2]National Oncology Institute of Maria Sklodowska-Curie – National Research Institute (branch in Gliwice)

Introduction

In the period of Covid-19 complications with the nature of neurological deficits, interest in Hyperbaric Oxygen Therapy (HBOT) was increased. The beneficial effects of this non-invasive method confirmed in clinical trials in other diseases were the driving force to study an impact of HBOT on the most lethal tumor of the central nervous system, which is glioblastoma multiforme (GBM).

A unique feature of glioblastoma are extensive foci of hypoxia that trigger the invasive phenotype. Hypoxia as a major cause of GBM recurrence by promoting cell migration, angiogenesis, edema, metabolic reprogramming, chemoresistance and tumor cold character is an attractive target for glioma therapy. Hovewer, an influence of HBOT on GBM is controversial because the results published so far are inconsistent. Therefore, the aim of our study was to assess the influence of HBOT on the selected malignant features of glioma cells.

Material and methods

Experiments were conducted on three human glioma cell lines (1) commercial T98G, (2) de novo patient derived HROG02 and (3) HROG17 recurrent glioma cell line. Cells cultured in hypoxic conditions (HypoxyLab) reflecting intratumor hypoxia (2.5%) were placed in hyperbaric oxygen chamber or exposed to ionizing radiation (10Gy) or Temozolomide action. Some cellular parameters were analyzed: mitochondrial activity / cell vitality (MTT), mitotic potential (Hoechst staining), mitochondrial membrane potential (Mitotracker), cell migration (wound healing assay), expression of HIF-1 alfa, MMP-2,9,14, TNF-alfa, IL-1beta (PCR), and sensitivity to temozolomide/radiotherapy.

Results

Our study shows the delayed and prolonged over time anti-glioma effect of HBOT for most of the tested parameters in commercial and primary cell lines including recurrent glioma. However, the potency of HBOT action was different for the studied cell lines and was dependent on the number of sessions and the time point when the analyzes were performed (time elapsed since the last session).

Conclusions

HBOT needs further research because as non invasive and multitarget therapy may become an important part of GBM treatment silencing its invasive potential, sensitizing glioma cells to chemotherapy and in the result, improving efficacy of standard therapy applied nowadays.

Key words: Glioma, HBOT, adjuvant therapy

Expression of clinically relevant drug transporters in proximal tubule cells from transplanted kidney in humans

J Łapczuk-Romańska , J Hybiak , M Marchelek-Myśliwiec , M Słojewski [4], E Urasinska , M Drozdzik
[1]Department of Experimental and Clinical Pharmacology, Pomeranian Medical University, Szczecin, Poland, [2]Department of Pathology, Pomeranian Medical University, Szczecin, Poland, [3]Department of Nephrology, Pomeranian Medical University, Szczecin, Poland, [4]Department of Urology, Pomeranian Medical University, Szczecin, Poland, [5]Department of Pathology, Pomeranian Medical University, Szczecin, Poland, [6]Department of Experimental and Clinical Pharmacology, Pomeranian Medical University, Szczecin, Poland

Introduction: Renal drug transporters, primarily located in basolateral and apical membrane of proximal tubule cells, play a pivotal role in secretion and reabsorption of drugs and endogenous substances. Recent studies have shown that renal diseases may affect the expression of transporters in kidney. However, the expression of these transporters in human transplanted kidney (with and without its rejection) has not been defined yet.
Aim of the study: The aim of this study was to analyze and compare gene expression of important renal ABC and SLC transporters in proximal tubule cells in transplanted kidney with and without acute rejection and healthy kidney in humans.
Material and methods: The expression of ABC and SLC transporters in proximal tubule cells from human normal kidney (n=8), transplanted kidney (n=7) and rejected transplanted kidney (n=8), dissected from tissue sample using laser microdissection system, were measured using quantitative reverse transcription polymerase chain reaction (rt-PCR). Differences between study groups were evaluated using the nonparametric Kruskal–Wallis test for multiple comparisons with post hoc Dunn's test. $p<0.05$ were considered significant.
Results: Of the analyzed ABC and SLC transporters, *ABCB5*, *ABCB11*, *ABCG5*, *ABCG8*, *SLC01A2*, *SLCO1B1*, *SLCO1B3*, *SLCO1C1* were not detected (mean Ct>35) in proximal tubules from the studied groups. Moreover, our study show that the expression of *SLC22A4*, *SLC22A6*, *SLC22A7*, *SLC22A8*, *SLC28A1*, *SLC47A1*, *SLC22A11*, *SLC15A2*, *SLC16A1*, *ABCC2*, *ABCC5*, *ABCC6* is suppressed while *SLC22A2*, *SLCO4A1* and *ABCB1* is significantly higher in proximal tubule cells from rejected transplanted kidney in comparison to control.
Conclusions: The obtained results suggest that the expression of important renal dug transporters changes in transplanted kidney rejection, which may lead to differences in the elimination of drugs in these patients.

Key words: drug transporters, human kidney, human renal allograft, renal allografts rejection, proximal tubule cells

---

New 1,3,4-oxadiazole derivatives of pyrrolo[3,4-d]pyridazinone improve intestinal barrier integrity in TNBS-induced colitis

Marta Szandruk-Bender [1], Beata Nowak [1], Anna Merwid-Ląd [1], Stanisław Dzimira [2], Łukasz Szczukowski [3], Piotr Świątek [3], Adam Szeląg [1]
[1]Department of Pharmacology, Wroclaw Medical University, J. Mikulicza-Radeckiego 2, 50-345 Wrocław, Poland, [2]Department of Pathology, Wroclaw University of Environmental and Life Sciences, C.K. Norwida 31, 50-375 Wrocław, Poland, [3]Department of Medicinal Chemistry, Wroclaw Medical University, Borowska 211, 50-556 Wrocław, Poland

Introduction: The intestinal mucosal barrier is a complex structure that separates the internal and lumen environments. This physical barrier consists of a single layer of epithelial cells connected by tight junctions and a mucus layer composed mainly of mucins produced by goblet cells. An impaired intestinal barrier may lead to excessive mucosal immune system activation, which in turn may contribute to the pathogenesis of intestinal diseases, including inflammatory bowel disease (IBD). Therefore, improving the integrity of the intestinal barrier may be a therapeutic approach to prevent or treat IBD. **Aim:** The current study aimed to assess the effect

of the new pyrrolo[3,4-*d*]pyridazinone derivatives on the expression of tight junction proteins and the content of goblet cells and mucus in colon tissues in TNBS-subjected rats. **Materials and Methods:** For this study were used colon tissues from the biobank. The original experiment was carried out on Wistar rats with TNBS-induced colitis. New pyrrolo[3,4-*d*]pyridazinone derivatives (compounds **7b**, **10b**, or **13b**) were given intragastrically for 16 consecutive days at 10 or 20 mg/kg doses. Colitis was induced by rectal administration of TNBS solution on day 15th, and on day 17th, rats were sacrificed, and colon tissues were collected. The control group received intragastrically and rectally proper vehicles. The colitis (TNBS) group received proper vehicle intragastrically and TNBS solution rectally. The expression of tight junction proteins (CLDN1, OCLN, ZO1) was quantified by ELISA assay. The goblet cells and mucus content were assessed by histopathological analysis of Alcian blue-stained colon tissue specimens using the 0-6 scale. For statistical analysis, ANOVA with Tukey's *post hoc* test was used. **Results:** Compounds **7b** and **13b** (20 mg/kg) counteracted the TNBS-induced decrease of CLDN1, OCLN, and ZO1 concentration ($p<0.001$ for **7b** in all cases; $p<0.05$, $p<0.01$, $p<0.01$ for **13b**, respectively). Compound **7b** (10 or 20 mg/kg) and compound **13b** (20 mg/kg) reverted the TNBS-caused destruction by increasing the number of goblet cells and amount of mucus ($p<0.01$, $p<0.001$, $p<0.01$, respectively). **Conclusions:** New pyrrolo[3,4-*d*]pyridazinone derivatives normalized the colonic expression of tight junction proteins and prevented goblet cells and mucus depletion in rats with experimental colitis, exerting a beneficial effect on mucosal epithelial barrier integrity.

Key words: experimental colitis, trinitrobenzenesulfonic acid, inflammatory bowel disease, intestinal barrier, tight junction proteins,pyrrolo[3,4-d]pyridazinone

---

Transport of antiepileptic and anticancer agents on the level of blood-brain barrier in vitro

Piotr Ruszkowski, Zuzanna Borkowska, Przemysław Mikołajczak
Poznan University of Medical Sciences, Department of Pharmacology

Introduction
Treatment of neurodegenerative diseases including brain tumors and correlated with them seizures, can be problematic. Anti-cancer drugs need to penetrate the blood-brain barrier and then reach therapeutic concentration in the brain tissue. The barrier is a specific cell membrane system built of various types of cells. Its main physiological function is to inhibit the permeation of toxic substances from the blood to the brain.
Material and methods
In this study we utilized in vitro model of the blood-brain barrier consisting of human derived endothelial cell line (hCMEC/D3) and normal human astrocytes cell line, in co-culture system. Before each experiment we measured the trans-endothelial electrochemical resistance (TEER) to confirm integration of the cell layers. The purpose of this experiment was to measure the permeation speed through the blood-brain barrier (BBB) of anticancer agent - temozolomide (TMZ) in combination with antiepileptic drug - lamotrigine (LTG).
Results
The studies have shown that TMZ is relatively well distributed across the BBB. The transport of TMZ through BBB occurs fastest at a concentration of 50 micromoles with the value of permeability coefficient 1,25x10-6 cm/sec. The penetration of the alkylating drug in the presence of antiepileptic agent was the fastest presenting permeability coefficient of 3,22x10-6 cm/sec.
Conclusion
The interaction between these two drugs can increase the effectiveness of the treatment of convulsions coexisting with brain tumors, in particular with glioblastoma multiforme. Furthermore we detected that the combination of these drugs in a lower micromolar concentrations penetrates the BBB much slower and is unable to achieve therapeutic effects in the central nervous system.

Key words: blood-brain barrier, cell lines, penetration, antiepileptic agents, anticancer agents

---

Effect of phloridzin on oxidative stress parameters in the lenses of rats with type 2 diabetes

Maria Zych [1], Katarzyna Urbisz [1], Magda Dejneka-Dynek [1], Nela Wilk [1], Weronika Borymska [1], Piotr Londzin [2], Joanna Folwarczna [2], Ilona Kaczmarczyk-Żebrowska [1]
[1]Department of Pharmacognosy and Phytochemistry, Faculty of Pharmaceutical Sciences in Sosnowiec, Medical University of Silesia, Katowice, [2]Department of Pharmacology, Faculty of Pharmaceutical Sciences in Sosnowiec, Medical University of Silesia, Katowice

Oxidative stress and non-enzymatic glycation are considered important factors of diabetic cataract development. Antioxidants, which include phloridzin, an apple polyphenol, may delay the loss of the lens transparency. Phloridzin is a prototype of new antidiabetic drugs – sodium-glucose cotransporter 2 inhibitors, including dapagliflozin and canagliflozin.
The aim of the study was to investigate the effect of phloridzin on oxidative stress parameters in the lenses of rats with experimentally induced type 2 diabetes (T2D). Dapagliflozin and canagliflozin were used as positive controls.
The study was conducted on female Wistar rats, which were divided into 6 groups: non-diabetic control, T2D control, T2D+phloridzin (20 or 50 mg/kg p.o.), T2D+dapagliflozin (1.4 mg/kg p.o.) and T2D+canagliflozin (4.2 mg/kg p.o.). T2D was induced by a high-fat diet and a single intraperitoneal injection of streptozotocin at a dose of 40 mg/kg. The administration of the tested substances started 1 week after the streptozotocin injection and lasted 4 weeks. The rats were euthanized, the lenses were isolated, and 10% (w/v) homogenates in phosphate buffered saline were prepared from them. The activity of superoxide dismutase (SOD), catalase and glutathione peroxidase, and the content of non-protein thiol groups (NPSH), substances reactive with thiobarbituric acid (TBARS), advanced oxidation protein products and advanced glycation end products (AGEs) were determined in the homogenates.
In the lenses of the control rats with T2D, an increase in the activity of SOD, a decrease in the NPSH content, and increases in the TBARS and AGEs content were observed compared to the non-diabetic control rats. Phloridzin at both doses did not cause statistically significant changes of the studied parameters in the lenses of rats with T2D. After administration of dapagliflozin or canagliflozin, a decrease in the SOD activity, an increase in the NPSH content, and decreases in the TBARS and AGEs content were observed in relation to the control rats with T2D.
In conclusion, phloridzin, unlike dapagliflozin and canagliflozin, did not exert beneficial effects on the oxidative stress parameters and the content of AGEs in the lenses of rats with T2D. It seems that phloridzin should not be considered useful in the prevention of diabetic cataract development.
This study was supported by the Medical University of Silesia (grant No. PCN-1-052/N/2/F).

Key words: phloridzin, dapagliflozin, canagliflozin, lenses, oxidative stress, type 2 diabetes, rats

---

Effects of maternal separation, lipopolysaccharide and their combination on fractalkine signaling in brain structures of male and female adolescent rats

Michał Zieliński, Miłosz Gołyszny, Ewa Obuchowicz
Department of Pharmacology, Faculty of Medical Sciences in Katowice, Medical University of Silesia, Medyków 18 Street, 40-752 Katowice, Poland

CX3CL1 (fractalkine), is predominantly expressed in central nervous system. CX3CL1-CX3CR1 axis is an important regulatory system for microglia activation and function. This signaling pathway is crucial for proper synapse-related processes, neurogenesis and development of neural networks during early brain development

(Arnoux et al., 2013; Sheridan et al., 2013). Early-life stressful events are considered as predisposing factors for mood and anxiety disorders later in life (Syed and Nemeroff, 2017).
The aim of this study was to investigate if maternal separation, lipopolysaccharide and combination of both factors affect expression of *CX3CL1* and *CX3CR1* in rat brain structures: olfactory bulb (OB), frontal cortex (FC), hippocampus (HIP), amygdala (AMY), pituitary (PT) and hypothalamus (HT).
Wistar rat pups (♂,♀) formed four experimental groups: untreated non-stressed control (C), untreated stressed (MS), non-stressed treated with LPS (LPS), stressed and treated with LPS (MS+LPS). Pups from MS and MS+LPS groups were subjected to the procedure of MS. On PND 42, rats received injection of LPS (LPS and MS+LPS group) or saline (control and MS group). Six hours after injection rats were sacrificed and brain structures were isolated. Quantitative RT-PCR was performed. Data were analyzed using multifactorial ANOVA. Differences between groups were analyzed by Student's t-test or Mann-Whitney test.
LPS caused highly significant decrease in *CX3CL1* expression in FC and HIP in both sexes. LPS induced more remarked decrease in *CX3CL1* expression in OB and HT in both sex of rats subjected to MS. In HIP its downregulation was strong but similar in LPS and MS+LPS groups in both sexes.
In females, but not in males MS markedly reduced its expression in AMY and HT. LPS and MS+LPS caused a significant decrease in *CX3CR1* expression in all studied brain structures in males and females, apart from FC in males. LPS alone or in combination with MS evoked stronger suppressive effects on *CX3CR1* expression in female than male rats.
Our data suggest that CX3CL1-CX3CR1 signaling is involved in long-term consequences of early life stress connected with activation of inflammatory system in rat brain because MS induced alterations in this pathway and enhanced effects of the subsequent immunostimulator challenge in some brain structures.
This work was supported by a statutory grant (KNW-2-0030/N/9/N) from the Medical University of Silesia, Katowice, Poland.

Key words: fractalkine, maternal separation, stress, brain

Novel 1,3,4-oxadiazole derivatives of pyrrolo[3,4-d]pyridazinone reveal no significant histological liver and kidney toxicity

Anna Merwid-Ląd [1], Piotr Ziółkowski [2], Łukasz Szczukowski [3], Piotr Świątek [3], Małgorzata Trocha [4], Adam Szeląg [1], Marta Szandruk-Bender [1]
[1]Department of Pharmacology, Wroclaw Medical University, J. Mikulicza-Radeckiego 2, 50-345 Wroclaw, Poland, [2]Department of Clinical and Experimental Pathology, T. Marcinkowskiego 1, 50-368 Wroclaw, Poland, [3]Department of Medicinal Chemistry, Wroclaw Medical University, Borowska 211, 50-556 Wroclaw, Poland, [4]Clinical Department of Diabetology and Internal Diseases, Wroclaw Medical University, Borowska 213, 50-556 Wroclaw, Poland

Introduction: Our previous studies confirmed that newly synthesized substances, 1,3,4-oxadiazole derivatives of pyrrolo[3,4-*d*]pyridazinone exerted anti-inflammatory and anti-nociceptive activities with reduced gastrotoxicity. They also alleviate the magnitude of the experimental colitis in rats.
Aim: The aim of the current project was to evaluate the impact of these compounds, named 7b, 10b, and 13b, on the liver and kidneys, two organs crucial for the metabolism and excretion of xenobiotics and very susceptible to drug-induced injury.
Materials and Methods: For histological evaluation, liver and kidney tissues from the biobank were used. The original experiment was conducted on Wistar male rats with TNBS-induced colitis. The studied substances were given intragastrically at 10 or 20 mg/kg doses for 16 consecutive days. Later, the colitis was induced by rectal administration of ethanol TNBS solution on day 15th and 48 h after the animals were sacrificed and tissues we collected. The negative control received intragastrically and rectally proper vehicle. A standard procedure for hematoxylin-eosin staining was performed, and samples were evaluated in 100x magnification.

Samples (N=8-12) from groups receiving the middle and the highest doses were chosen for assessment of tissue toxicity because the lowest dose of the studied compounds revealed insignificant action on the colitis outcome. Results: Independent from the studied group, the microscopic evaluation of kidneys revealed no significant pathological changes and was almost the same in all samples. The only significant feature was hyperemia in all specimens. In the control and the studied groups, there were no features of kidney atrophy, degeneration, inflammation, renal papillary necrosis, or interstitial nephritis. Similarly to the kidneys, hyperemia was the only significant change in all studied liver specimens. No liver cell injury or atrophy characteristics were noticed independently from the studied group. Small lymphocytic clusters observed in the portal spaces should be considered an element of the normal histological image.
Conclusions: Based on the histological images, it may be stated that newly developed substances 7b, 10b, and 13b, after 16 days of administration, did not cause a significant tissue injury to the kidneys or liver.

Key words: pyrrolo[3,4-d]pyridazinone derivatives, histology, kidney, liver

---

Drug transporters in pharmacotherapy

Marek Droździk
Department of Experimental and Clinical Pharmacology, Pomeranian Medical University, Szczecin, Poland

Drug transport proteins are divided into two major superfamilies, i.e., ATP-binding cassette transporters (ABC, comprising about 50 members, classified into 7 families) and solute carriers (SLC, with more than 400 membrane proteins allocated into over 60 families). Transporters of ABC-superfamily are mainly engaged in efflux activities, and the list of major representatives includes multidrug resistance protein 1/P-glycoprotein (MDR1, P-gp, *ABCB1*), multidrug resistance-associated protein 2 (MRP2, *ABCC2*), 3 (MRP3, *ABCC3*) and 4 (MRP4, *ABCC4*) as well as breast cancer resistance protein (BCRP, *ABCG2*). SLC carriers mediate cellular influx and/or efflux of substrate molecules, operating in gradient-dependent mode. Organic anion transporter 1 (OAT1, *SLC22A6*), 2 (OAT2, *SLC22A7*), 3 (OAT3, *SLC22A8*), 4 (OAT4, *SLC22A11*), organic cation transporter 1 (OAT1, *SLC22A1*), 2 (OAT2, *SLC22A2*), 2 (OAT, *SLC22A3*), organic cation/carnitine transporter family members OCTN1 (*SLC22A4*) and OCTN2 (*SLC22A5*), multidrug and toxin extrusion proteins (MATE1/*SLC47A1*; MATE2/*SLC47A2*; MATE2-K/*SLC47A2*), peptide transporters 1 and 2 (PEPT1/*SLC15A1*; PEPT2/*SLC15A2*), equilibrative nucleoside transporters 1 (ENT1/*SLC29A1*) and 2 (ENT2/*SLC29A2*) and urate transporter 1 (URAT1/*SLC22A12*) belong to the major representatives of SLC carriers. Coordinated function of the uptake and efflux transporters mediate vectorial transport of molecules across cell membranes, e.g. in the gastrointestinal tract, liver or kidneys. Pathological states affecting organ function (e.g. inflammatory bowel disease, hepatitis, cholestatic liver diseases, kidney failure) impact drug transporter levels and function, and thus contribute to altered pharmacokinetics and drug actions. Information about drug transporters in mandatory to tailor drug pharmacotherapy in specific groups of patients.

Key words: drug transporters, pharmacotherapy, ABC, SLC

An injectable peptide MOTS-c: for muscle regeneration or sport doping purpose?– an in vitro study

Natalia Leciejewska [1], Paweł A. Kołodziejski [1], Ewa Pruszyńska- Oszmałek [2], Oskar Sosiński [2], Maciej Sassek [2]

[1]*Department of Animal Physiology, Biochemistry and Biostructure; University of Life Sciences Poznań,*
[2]*Department of Animal Physiology, Biochemistry and Biostructure, University of Life Sciences Poznań*

Introduction: MOTS-c is a bioactive and exercise-induced peptide of 16 amino acids which is encoded in a mitochondrial open reading frame of the 12S rRNA-c gene. It is a peptide with a high therapeutic potential - so far animal studies have shown an improvement in insulin sensitivity, affects the body's energy balance and inhibits the development of obesity in mice. It would seem that the work on the MOTS-c is only experimental, but it is already available in online sales portals as a performance-enhancing drug. Although a new tool has been developed to detect MOTS-c in human plasma samples, this peptide is marketed and labeled as a dietary supplement or contains statements such as “Not for human consumption” or “Research use only.” MOTS-c available for sale online is offered in the injectable form. In our study, we decided to check whether MOTS-c affects the processes related to the regeneration of skeletal muscle cells by affecting differentiation.
Material and methods: The material was a murine skeletal cell line C2C12. Using three doses of MOTS-c and based on molecular biology techniques we tested: cell survival (MTT assay), changes in the expression of transcription factors as markers of muscle differentiation as well as ERK1/2 phosphorylation (qPCR and Western Blot techniques). We also assessed lipid accumulation with stearic, palmitic and oleic fatty acids using ORO staining method. The methods of calculation were unpaired Student’s t-test (two-tailed distribution) or one-way analysis of variance (ANOVA) with a Dunnett post hoc test.
Results: We found that MOTS-c increases the survival of muscle cells at 10 and 100 nM ($p < 0,01$). MOTS-c stimulates also in immature myocytes the phosphorylation of ERK1/2 kinase - a strong stimulator of processes related to proliferation ($p < 0,05$). We showed an increase in the expression of muscle-regulating factors: myogenin and myoD after 2 and 6 days of differentiation. Moreover, we showed that MOTS-c in 100 nM reduces intracellular lipid accumulation after incubation with free-fatty acids thus improving the metabolic status of cells ($p<0,01$).
Conclusions: Collectively, our study and other literature reports provide evidence that MOTS-c may be a promising agent in the treatment of skeletal muscle while being a potentially abused substance in sports.
Acknowledgments: This study was financed by the National Science Centre No. 2018/31/D/NZ4/01121

Key words: muscle regeneration, performance-enhancing drug, doping, novel peptides

---

Synovial membrane and Hoffa’s Pad solute carrier transporters in patients with rheumatoid arthritis

D Malinowski [1], N Komaniecka [2], S Maroszek [2], K Piotrowska [3], A Pawlik [3], M Droździk [2]
[1]Department of Pharmacokinetics and Therapeutic Drug Monitoring, Pomeranian Medical University, Szczecin, Poland, [2]Department of Experimental and Clinical Pharmacology, Pomeranian Medical University, Szczecin, Poland, [3]Department of Physiology, Pomeranian Medical University, Szczecin, Poland

Rheumatoid arthritis (RA) is the most common immune-mediated disease with a strong genetic influence that leads to joint destruction. A number of immune cells that affect joint tissues are involved in the pathogenesis of this disease, leading to synthesis of many pro-inflammatory mediators. The transport of drugs, as well as many cytokines and chemokines involved in the development of inflammation in RA patients, is mediated by membrane transporters. Membrane transporters are proteins that mediate the transfer of substrates across biological membranes. To date there are no studies examining the expression of SLC transporters in joint tissues. The aim of the study was to evaluate the expression of individual SLC family transporters in the synovial membranes and periarticular fatty tissue (Hoffa’s pad) of RA patients.
The study included 20 patients with rheumatoid arthritis and 20 patients with osteoarthritis as the control group who were undergoing joint replacement surgery as a normal part of clinical care. Quantitative real-time PCR was performed using individual gene expression assays for mRNA expression analysis. Immunohistochemistry analysis was performed for immunoreactive score calculations.
In the synovial membranes and Hoffa’s pad of RA patients the following 17 membrane transporters (out of 35 total) were defined at relevant expression levels for SLC transporter superfamily: SLC15A2, SLC16A3, SLC19A1, SLC2A9, SLC22A1, SLC22A3, SLC22A4, SLC22A5, SLC22A18, SLC33A1, SLC47A1, SLC51A, SLC7A5, SLC7A6, SLC01C1, SLC02B1, SLC04A1. The confirmed expression of these transporters in the

synovial membranes as well as Hoffa's pad of patients with RA and OA, and the differences in their expression between these groups, suggests the involvement of SLC transporters in both the maintenance of homeostasis under physiological conditions in the tissues of the joints, as well as in the inflammatory process in RA.

Key words: drug transporters, SLC, Hoffa's pad, synovial membrane, rheumatoid arthritis

---

Icariin - effect on oxidative stress parameters in testes and sex hormones in serum of rats with type 2 diabetes

Katarzyna Szałabska-Rąpała [1], Weronika Borymska [2], Maria Zych [2], Ilona Kaczmarczyk-Żebrowska [2]
[1]Doctoral School of the Medical University of Silesia in Katowice, Discipline of Pharmaceutical Sciences, Department of Pharmacognosy and Phytochemistry, Faculty of Pharmaceutical Sciences in Sosnowiec, Medical University of Silesia, Katowice, [2]Department of Pharmacognosy and Phytochemistry, Faculty of Pharmaceutical Sciences in Sosnowiec, Medical University of Silesia, Katowice

In type 2 diabetes mellitus (T2DM), a state of disturbance of carbohydrate, lipid and protein metabolism is observed. Chronic hyperglycemia is accompanied by excessive production of free radicals and oxidative stress (OS), which can lead to numerous diabetic complications, including micro- and macrovascular changes, nephropathy, retinopathy, neuropathy or hormonal disorders. Icariin (IC) is a prenylated flavonoid that can be found in the herb of plants of the genus *Epimedium*. IC is believed to have antioxidant, cardioprotective or neuroprotective properties, and also to prevent erectile dysfunction.
The aim of this study was to investigate the effect of IC on the OS parameters in the testes of rats with experimentally induced T2DM as well as on the level of sex hormones in the serum.
The experiment was carried out on 4 groups of mature male Wistar rats: control non-T2DM rats, control T2DM rats and T2DM rats receiving IC orally at the doses of 5 or 25 mg/kg. T2DM was induced by high fat diet and single intraperitoneal injection of streptozotocin (40 mg/kg). The following OS parameters were investigated using spectrophotometric methods in homogenized rat testes to evaluate the impact of IC on it: soluble protein (SP), glucose, glutathione peroxidase (GPx), catalase (CAT), superoxide dismutase (SOD), non protein thiol groups (NPSH), total thiol groups, total oxidant status (TOS), total antioxidant response, thiobarbituric acid reactive substances and advanced oxidation protein products (AOPP). Serum estradiol and testosterone levels were estimated using ELISA method. The body weight and testicular weight of the rats were also recorded.
The results obtained in the course of the study indicate that T2DM led to a significant deterioration of parameters related to OS and to a decrease in the concentration of sex hormones. Both doses of IC resulted in a statistically significant fall of SOD and GPx activity. The dose of 5 mg/kg resulted also in a statistically significant reduction in CAT activity and a decrease in TOS level. The dose of 25 mg/kg resulted in additional increase in SP and NPSH levels, as well as a decrease in AOPP level. The level of sex hormones did not return to values comparable to the control group without T2DM after both doses of IC.
IC has a beneficial effect on the improvement of some parameters related to OS, but not on hormonal imbalances in T2DM rats.
This study was supported by the Medical University of Silesia (grant No. PCN-2-028/N/2/F).

Key words: icariin, type 2 diabetes, oxidative stress, male reproductive system disorders

---

Diabetes and the male reproductive system disorders - can plant neolignans help in combating its complications?

Katarzyna Szałabska-Rąpała [1], Weronika Borymska [2], Maria Zych [2], Ilona Kaczmarczyk-Żebrowska [2]
[1]Doctoral School of the Medical University of Silesia in Katowice, Discipline of Pharmaceutical Sciences, Department of Pharmacognosy and Phytochemistry, Faculty of Pharmaceutical Sciences in Sosnowiec, Medical University of Silesia, Katowice, [2]Department of Pharmacognosy and Phytochemistry, Faculty of Pharmaceutical Sciences in Sosnowiec, Medical University of Silesia, Katowice

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disease characterized by hyperglycemia (HG), in which there is an increase in the production of free radicals (FR) and the occurrence of oxidative stress (OS). It is also accompanied by disorders of lipid and protein metabolism, as well as hormonal imbalance. Long-lasting OS consequently leads to the occurrence of macro- and microvascular diseases in the organism, which may result in pathogenesis of male reproductive system (MRS) disorders like erectile dysfunction, histopathological changes in the testes, impaired sperm parameters or finally infertility.
Honokiol (HON) and magnolol (MAG) are polyphenolic compounds from the group of neolignans that can be found in the extract obtained from the bark of magnolia. These compounds are believed to have antioxidant, anti-diabetic or anti-inflammatory properties. Considering that the pathogenesis of diabetic complications in the MRS is mainly caused by HG, excessive production of FR and OS, these neolignans can serve as support in MRS disorders.
The therapeutic potential of neolignans was investigated in a study conducted on mature male Wistar rats. In addition to the healthy control and diabetic control groups, the study included groups receiving HON or MAG orally at the doses of 5 or 25 mg/kg, respectively. T2DM was induced by streptozotocin and high fat diet. The subject of interest in the study was the examination and comparison of the level of basic biochemical parameters and endogenous antioxidants, the antioxidant enzyme activity, sumaric parameters for OS and oxidative damage in the isolated and homogenized testes, as well as serum sex hormones levels of rats.
T2DM worsened the parameters related to OS and caused an imbalance of sex hormones. Administration of HON resulted mainly in the improvement of sumaric parameters for OS. In the case of MAG, both doses improved most biochemical markers related to antioxidant enzyme activity, sumaric parameters for OS and oxidative damage, as well as the higher dose improved additionally the level of endogenous antioxidants. Serum sex hormones level did not return to comparable values with the healthy control group.
The above results may indicate a beneficial effect of neolignans, especially MAG, on the parameters of OS and reduction of FR, but not on sex hormone imbalance, in rats with T2DM.
This study was supported by the Medical University of Silesia (grant No. PCN-2-007/N/2/F and No. PCN-2-028/N/2/F).

Key words: honokiol, magnolol, type 2 diabetes, oxidative stress, male reproductive system disorders

---

Antinociceptive effects of a new N-substituted 1H-isoindole-1,3(2H)-dione derivative with cyclooxygenase inhibitory activity

Anna Dziubina , Dominika Szkatuła , Jacek Sapa
[1]Jagiellonian University Medical College, Faculty of Pharmacy, Department of Pharmacodynamics, Medyczna 9, 30-688 Krakow, Poland, [2]Wrocław Medical University, Department of Medicinal Chemistry, Borowska 211, 50-556 Wroclaw, Poland, [3]Jagiellonian University Medical College, Faculty of Pharmacy, Medyczna 9, 30-688 Krakow, Poland

Introduction
Isoindoline-1,3-dione, also known as phthalimide derivatives have diverse bioactivities and are attractive molecules for drug discovery. The phthalimide ring does not cause side effects compared to the glutarimide moiety featured in certain drugs, such as thalidomide. Several reports demonstrated their the anti-microbial, anti-cancer, anti-convulsant, anti-inflammatory, analgesic and potential anticholinesterase activity.
The aim of our study was to evaluate analgesic and anti-oedematous activities of the new N-substituted 1H-isoindole-1,3(2H)-dione compound with affinity for both isoforms of cyclooxygenase.

Material and methods
The experiment were carried out on male Albino-Swiss mice (CD-1) and Wistar rats. Antinociceptive activity was examined in the hot plate test, the formalin test and capsaicin-induced pain model in mice. In a carrageenan-induced inflammatory (edema) model in rats, the anti-edematous and analgesic effects on thermal hyperalgesia and mechanical allodynia were evaluated. Additionally, the influence of the compounds on locomotor activity and motor coordination was assessed too.
Results
The tested compound increased the latency of the pain reaction in the hot plate test, inhibited the pain reaction induced by capsaicin and, comparable to meloxicam, reduced the pain reaction in both phases of the formalin test. After administration of carrageenan, it showed an anti-edematous and weak analgesic effect compared to meloxicam. In active, analgesic doses, it did not impair motor coordination and did not have a sedative effect.
Conclusions
Based on the tests carried out, it can be concluded that the tested compound has an analgesic effect of central and/or peripheral origin, with a weak anti-edematous effect. The involvement of TRPV1 receptors in the mechanism of analgesic action is considered, too. The obtained results revealed that the new 1H-isoindole-1,3(2h)-dione derivative has the analgesic and anti-inflammatory activity.
This study was financially supported by the Jagiellonian University Medical College grants No.:N42/DBS/000325.

Key words: phthalimide, formalin test, cyclooxygenase inhibition, anti-edematous activity

---

Bones: Interesting Targets for Drugs, Risk Factors, and Food Ingredients

Radoslav Omelka, Vladimira Mondockova, Veronika Kovacova, Martina Babikova, Nina Zemanova, Roman Biro, Monika Martiniakova
Faculty of Natural Sciences and Informatics, Constantine the Philosopher University in Nitra, Slovak Republic

Bones are highly adaptive, dynamic and metabolically active organs responsible for important mechanical and non-mechanical functions. They provide the basis for movement, store minerals, including potentially toxic ones, and support haematopoiesis. Additionally, bone serves as an endocrine organ that actively controls mineral metabolism and energy homeostasis. Together, these properties predetermine bones as interesting targets for different types of compounds. During our research, the effects of risk element cadmium (Cd), various food ingredients and products, specifically ethanol, acrylamide, cola beverages, amygdalin, as well as drugs and dietary supplements such as calcium (Ca), vitamins D3 (VD3) and K2 (VK2) on microstructural, cellular and molecular bone parameters were investigated. Subchronic exposure to Cd induced an early stage of osteoporosis and was associated with a reduced bone vascularization in the cortical bone of rats. In addition, Cd had an adverse impact on viability, morphology and function of cultivated osteoblasts. Subacute ethanol administration decreased relative volume (BV/TV), bone mineral density in the cortical bone and increased blood supply in primary osteons (PO). Acrylamide exposure elevated bone remodelling and was consistent with a higher density of secondary osteons and vasoconstriction in PO. However, after simultaneous administration, the adverse effects of ethanol were partially reduced by co-administration of acrylamide in mice. Long-term cola intake did not cause evident pathological alterations in the femoral bone microstructure and mechanical properties of adult mice, possibly due to a balanced diet and no restriction of physical activity. Short-term application of amygdalin negatively affected cortical bone microstructure including vascularization and parameters related to biomechanical properties in rabbits. In vitro analysis showed reduced matrix mineralization, increased bone resorption and decreased viability of amygdalin-treated osteoblasts. The impact of natural Ca, VD3 and VK2 on bone microstructure was analysed in a rat model of osteoporosis. The triple application of Ca, VD3, VK2, as well as simultaneous administration of Ca with VD3 increased trabecular BV/TV, trabecular bone surface, and effectively inhibited bone loss. In summary, our research revealed the influence of different substances on bone structure with various effects. The study was supported by KEGA 034UKF-4/2022, KEGA 012UKF-4/2023.

Key words: bone microstructure, therapeutics, risk factors, food ingredients, diseases, animal models

The Impact of Cemtirestat Treatment on Bone Parameters Reflecting Bone Quality in Rats with Experimentally Induced Diabetes

Monika Martiniakova [1], Veronika Kovacova [1], Vladimira Mondockova [1], Karol Svik [2], Piotr Londzin [3], Joanna Folwarczna [3], Marta Soltesova Prnova [2], Radoslav Omelka [1]
[1]Faculty of Natural Sciences and Informatics, Constantine the Philosopher University in Nitra, Slovak Republic, [2]Institute of Experimental Pharmacology and Toxicology, Slovak Academy of Sciences, Slovak Republic, [3]Faculty of Pharmaceutical Sciences in Sosnowiec, Medical University of Silesia in Katowice, Sosnowiec, Poland

Diabetes mellitus (DM) as a complex metabolic disorder is characterized by chronic hyperglycaemia caused by insulin resistance, impaired insulin secretion, or both. The most common classifications include type 1 DM (T1DM) and type 2 DM (T2DM). DM is often associated with many serious macrovascular and microvascular complications. Diabetic bone disease generally represents an important microvascular complication of DM. It is mainly manifested by poor bone quality and increased risk of fractures. Aldose reductase (AR), the first enzyme of the polyol pathway, has a primary role in the cascade of metabolic imbalances responsible for deleterious effects of hyperglycemia and is therefore considered an important therapeutic target. Many AR inhibitors have been developed as potential therapeutics for several diabetic complications. Cemtirestat (CMTI), a bifunctional drug acting as an AR inhibitor with antioxidant ability, has a neuroprotective impact that has already been demonstrated in experimental rat models of diabetes. The aim of our study was to examine the effects of CMTI treatment on femoral bone parameters reflecting bone quality in streptozotocin (STZ)-induced diabetic rats (appropriate animal model for T1DM) as well as in Zucker diabetic fatty (ZDF) rats, serving as a suitable animal model for T2DM. In STZ-induced diabetic rats, CMTI supplementation reduced only plasma triglyceride level, the other biochemical parameters did not differ significantly. Microcomputed tomography (micro-CT) revealed that all measured parameters characterizing trabecular bone mass and microarchitecture, as well as cortical microarchitecture and geometry were not affected by CMTI administration. In addition, CMTI supplementation had an insignificant effect on all investigated parameters related to bone mechanical properties. In ZDF-rats, biochemical analysis revealed no effect of CMTI treatment on the levels of plasma glucose, insulin, glycated haemoglobin, cholesterol, triglycerides, Ca, P, Mg. With the exception of increased trabecular thickness, all micro-CT parameters were not influenced by CMTI administration. Considering bone mechanical properties, no significant effect of CMTI treatment was reported between untreated and treated ZDF rats. In conclusion, insufficient effect of CMTI treatment on impaired bone quality in both T1DM and T2DM does not support its use in the therapy of this diabetic complication. The study was supported by VEGA 1/0444/20, VEGA 1/0416/22.

Key words: diabetes mellitus, cemtirestat, aldose reductase, bone quality, streptozotocin-induced diabetic rats, Zucker diabetic fatty rats

Evaluation of Heterobasidion annosum extract and 5-fluorouracil effects on general condition of mice in the colorectal cancer xenograft model

Anna Sadowska [1], Diana Sawicka [1], Ewa Zapora [2], Halina Car [1]
[1]Department of Experimental Pharmacology, Medical University of Bialystok, Szpitalna 37, 15-295 Bialystok, Poland, [2]Department of Silviculture and Forest Use, Institute of Forest Sciences, Bialystok University of Technology, Wiejska 45E, 15-351 Bialystok, Poland

Heterobasidion annosum (Fr.) Bref. sensu lato (s.l.) (HA) is one of the most destructive pathogens on conifer forest trees in the northern and temperate regions. Yet, a few thousand years ago in Traditional Chinese

Medicine emerged the idea of fungal treatment. Nowadays, mushrooms are considered a very important element in the prevention and treatment of many diseases. Various studies suggest that medicinal mushrooms may be supportive of our health by affecting the immune system or inflammatory responses, and through this also have anticancer effects, due to their biological compounds such as β-glucans, lectins, or triterpenes. It is expected that medicinal mushrooms would play an important role in the development of innovative agents without such severe side effects. In our previous studies, the antitumor effect of HA extract against colon cancer has been observed in *in vitro* and *in vivo* models.
5-Fluorouracil (5FU) is a cytostatic drug that is widely used in the therapy of gastrointestinal tract, breast, or head and neck cancer. Apart from the well-known toxic effects of 5FU directed to solid tumors, side effects such as myelosuppression, mucositis, diarrhea, nausea and vomiting, and cardiotoxicity have been observed. Therefore, the present study aimed to evaluate the influence of 5FU and HA extract on the general condition of mice, including blood morphology, or body weight changes. Mice with tumors were given HA extract and/or 5FU. Parameters such as body mass, food and water intake, and physical activity were controlled during the whole experiment. The morphological examination of blood was assessed using the ABCvet system. Statistical analysis was performed using GraphPad Prism version 10.
The results obtained did not reveal statistical changes in the body mass of mice during the experiment suggesting that the administration of tested extracts alone or together with the reference drug did not impair their general condition in the tested range. Analysis of blood samples showed significantly reduced levels of white blood cells (WBC) in the group of mice receiving 5FU. A slight reduction of WBC count was also noted in groups receiving extract together with 5FU compared to groups receiving only the extract.
The results suggest that the use of HA extract might have a more beneficial effect on the condition and health of mice in comparison to 5FU.
This work was financially supported by Medical University of Bialystok grants no. B.SUB.23.258 and B.SUB.23.215.

Key words: Heterobasidion annosum; colorectal cancer; 5 fluorouracil

---

Polymeric nanoparticles containing ketoester-based block and cholesterol moiety as a vehicle for doxorubicin delivery to breast cancer cells.

Katarzyna Niemirowicz-Laskowska [1], Paweł Misiak [2], Przemysław Wielgat [3], Karolina H. Markiewicz [2], Iwona Misztalewska-Turkowicz [2], Agnieszka Z. Wilczewska [2], Halina Car [1]
[1]Department of Experimental Pharmacology, Medical University of Bialystok, Szpitalna 37, 15-295 Bialystok, Poland, [2]Faculty of Chemistry, University of Bialystok, Ciolkowskiego 1K, 15-245 Bialystok, Poland, [3]Department of Clinical Pharmacology, Medical University of Bialystok, Waszyngtona 15A, 15-274 Bialystok, Poland

In 2020 female breast cancer has surpassed lung cancer and become the most commonly diagnosed cancer worldwide, as well as the leading cause of death in women. Doxorubicin (DOX) is an organic compound from the anthracycline group and is the most widely used drug in the treatment of breast cancer. DOX-based chemotherapy, although effective, is associated with serious side effects, including irreversible cardiotoxicity and myelosuppression. In effect, the aforementioned life-threatening adverse effects that were responsible for the prolonging of the patients' recovery and significantly rising medical expenses, have prompted the need to create a new and safer option of anticancer therapy that engage smart drug delivery systems.
Herein we report the in-depth biological evaluation of doxorubicin drug delivery systems including several block and statistical copolymers, composed of ketoester derivative, N-isopropylacrylamide, and cholesterol part, being in polymeric nanoparticles form (PNPs). The biocompatibility with representatives of host cells such as red blood cells (RBC), monocytic cells (THP-1), and cardiomyocyte cells (H9c2 2-1) was tested. Cytotoxic activity and mode of action against estrogen-dependent MCF-7 breast cancer cells were investigated. For the above mentioned purpose spectrophotometric, fluorometric and luminometric techniques were engaged.

Results have shown that DOX-loaded PNPs show high efficacy against estrogen-dependent MCF-7 breast cancer cell line despite low doses of DOX applied and good compatibility with normal cells. Research confirms the ability of PNPs to insert into the plasma membrane and to increase in ROS production. The above mentioned mechanisms might be responsible for observed cell death. In effect, created PNPs are attractive candidates as a DOX carriers, however further studies involving in vivo models, are required to select a great candidate for DOX delivery.

Key words: polymeric nanoparticles, drug delivery systems, breast cancer, doxorubicin

---

Influence of N-steroidal imidazolium salts (C6-C10) on breast cancer cells

Halina Car , Diana Sawicka , Marta Malinowska [3], Izabela Jastrzębska [3], Anna Sadowska , Beata Skonieczna
[1]Department of Experimental Pharmacology, Faculty of Health Sciences, Medical University of Bialystok, Szpitalna, 37, 15-295 Białystok, Poland;, [2]Department of Experimental Pharmacology, Faculty of Health Sciences, Medical University of Bialystok, Szpitalna, 37, 15-295 Białystok, Poland;, [3]Department of Organic Chemistry, Faculty of Chemistry, University of Bialystok, Ciołkowskiego, 1K, 15-245 Białystok, Poland, [4]Department of Experimental Pharmacology, Faculty of Health Sciences, Medical University of Bialystok, Szpitalna, 37, 15-295 Białystok, Poland;, [5]Department of Experimental Pharmacology, Faculty of Health Sciences, Medical University of Bialystok, Szpitalna, 37, 15-295 Białystok, Poland;

In 2020, there were 2.3 million women diagnosed with breast cancer and 685 000 deaths globally. The development of new therapeutics in medicinal chemistry is based on the synthesis of a new biological compounds, including by combining bioactive molecules, which could be used in anti-cancer therapy. Imidazolium salts contain an aromatic five-membered ring in their structure exhibit relevant biological functions. We synthesized imidazolium salts with N-steroid (3-oxo-23,24-dinorchol-4-en-22-al) differing in the number of carbon atoms in the alkyl chain **(C6-C10).**
The aim of present study was to evaluate the anticancer potential of N-steroidal imidazolium salts **(C6-C10)** against estrogen- **(MCF-7)** and non-estrogen-dependent **(MDA-MB-231)** breast cancer cells. To evaluate the cytotoxicity of imidazolium salts in concentrations (5-100 μg/mL) MTT assay was used. Statistical analysis was performed using GraphPad Prism 9.

The results showed significant reduction of the number of tested breast cancer cells after 24h of incubation with N-steroid salts used at different range of concentrations (5-100 μg/mL). The strongest cytotoxic effect against the tested breast cancer lines was found for the salt **KC_Okt (-C8H17)**. The tested salt reduced the cell viability of the MCF-7 line to **51%** and MDA-MB-231 line to **49.9%.**
In conclusion, N-steroid imidazolium salts **(C6-C10)** depends on the structure of the tested salts exhibit cytotoxic effects against estrogen- and non-estrogen-dependent breast cancer cells.
This work was financially supported by the Medical University of Bialystok B.SUB.23.215 (HC) and B.SUB.23. 206 (DS). The authors would like to thank to the Department of Organic Chemistry, University of Bialystok for the synthesis of the tested salts.

Key words: N-steroidal salts, breast cancer

Evaluation of the anticancer potential of derivatives N-steroidal imidazolium salts

Diana Sawicka , Marta Malinowska [2], Izabela Jastrzębska [2], Anna Sadowska , Beata Skonieczna , Halina Car
[1]Department of Experimental Pharmacology, Faculty of Health Sciences, Medical University of Bialystok, Szpitalna, 37, 15-295 Białystok, Poland;, [2]Department of Organic Chemistry, Faculty of Chemistry, University

of Bialystok, Ciołkowskiego, 1K, 15-245 Białystok, Poland, [3]Department of Experimental Pharmacology, Faculty of Health Sciences, Medical University of Bialystok, Szpitalna, 37, 15-295 Białystok, Poland;, [4]Department of Experimental Pharmacology, Faculty of Health Sciences, Medical University of Bialystok, Szpitalna, 37, 15-295 Białystok, Poland;, [5]Department of Experimental Pharmacology, Faculty of Health Sciences, Medical University of Bialystok, Szpitalna, 37, 15-295 Białystok, Poland;

The main goal of medicinal chemistry is to improve the biological properties of compounds, including by combining bioactive molecules and for use in a cancer therapy. Breast cancer is the second leading cause of cancer deaths worldwide, contributing to 11.6% of total cancer deaths.
Imidazolium salts are imidazole derivatives in which nitrogen atoms were substituted with alkyl or aryl groups showing important biological functions, for eg. antifungal and antibacterial. Imidazolium salts were synthesized with one of the substituents being a steroid derivative and the other being a simple hydrocarbon chain differing in the number of carbon atoms **(C1-C5)**.
The aim of the studies was to evaluate the anticancer potential of tested salts against estrogen- **(MCF-7)** and non-estrogen-dependent **(MDA-MB-231)** breast cancer cells was determined. To evaluate the cytotoxicity of tested salts the MTT assay was used. Statistical analysis was performed using GraphPad Prism 9.
The results showed that tested N-steroid salts contained different carbon atoms **(C1-C5)** number administered at 5-100 μg/ml concentrations significantly reduced the number of tested breast cancer cells after 24h incubation. The strongest cytotoxic effect against the tested breast cancer lines was found for the salt **KC_Bu (-C4H9)**. The tested salt reduced the cell viability of the MCF-7 line to **55.7%** and MDA-MB-231 line to **50.9%.**
In conclusion, N-steroid imidazolium salts exhibit cytotoxic effects against estrogen- and non-estrogen-dependent breast cancer cells. The observed effect depends on the structure of the tested salts.
This work was financially supported by the Medical University of Bialystok B.SUB.23. 206 (DS) and B.SUB.23.215 (HC). The authors would like to thank to the Department of Organic Chemistry, University of Bialystok for the synthesis of the tested salts.

Key words: imidazolium salts, breast cancer

Polymeric nanocarriers for neuroprotective drug delivery to central nervous system

Władysław Lasoń [1], Magdalena Regulska [1], Magdalena Procner [1], Monika Leśkiewicz [1], Marta Szczęch [2], Beata Grygier [1], Agnieszka Basta-Kaim [1], Krzysztof Szczepanowicz [2], Piotr Warszyński [2]
[1]May Institute of Pharmacology, Polish Academy of Sciences, [2]Jerzy Haber Institute of Catalysis and Surface Chemistry, Polish Academy of Sciences

Introduction
Despite an enormous progress in understanding molecular basis of age-related neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease, no efficient neuroprotective strategy has been invented so far. Potential neuroprotectants often display unfavourable pharmacokinetic properties, poor water solubility, instability in organism fluids, and a weak penetration to the central nervous system through blood-brain-barrier (BBB). Furthermore, they may cause peripheral toxicity and other undesired effects. Recently, nanotechnology provided some tools to overcome these obstacles. Among them, polymeric nanocarriers are very promising because they allow payload flexibility and easy surface modifications.
Aim
The aim of our project was to estimate biocompatibility and ability to cross BBB of polymeric-based nanoparticles designed as carriers of hydrophobic neuroprotective substances.
Material and methods
The potential cytotoxic effects of nanocarriers were estimated in differentiated and undifferentiated human neuroblastoma SH-SY5Ycell lines using WST-1 and LDH tests. Next, biocompatibility of the polymeric nanocarriers was additionally evaluated in organotypic hippocampal culture (OHC) exposed to oxygen-glucose

deprivation (OGD). Ability of the rhodamine-labeled nanoparticles to cross BBB was studied using hCMEC/D3 cell line.

Results

Results showed that the new designed polymeric nanocarriers abbreviated as AOT/$(PLL/PGA)_2$-g-PEG and PCL/$(PLL/PGA)_2$-g-PEG, affected viability of SH-SY5Y cells only when used in the highest concentrations, and had no damaging effect on OHC. Of note, in some concentrations they even decreased the OGD-elevated proinflammatory cytokine level in OHC. Both types of nanoparticles crossed BBB in its *in vitro* model in time-dependent manner.

Conclusions

Overall, these data point to biocompatibility and potential utility of polymeric-based nanoparticles for transporting neuroprotective substances to central nervous system.

Acknowledgements

The authors thank the National Science Centre in Poland for financial support (Grant no. 2019/34/H/ST5/00578).

Key words: nanocarriers, neuroprotection, biocompatibility, blood-brain barrier, cell lines

---

IMPLICATIONS OF NOP RECEPTOR SYSTEM IN SOCIABILITY IMPAIRMENTS UNDER MIGRAINE CONDITION

Akanksha Mudgal [1], Olga Wronikowska-Denysiuk [2], Darian Peters [3], Isabel Snow [3], Madeline Martinez [3], Lawrence Toll [3], Akihiko Ozawa [3], Katarzyna Targowska-Duda [1]

[1]Charles E. Schmidt College of Medicine, Florida Atlantic University, Boca Raton, FL; Department of Bio pharmacy Medical University of Lublin Chodzki Str. 4, 20-093 Lublin, Poland, [2]Department of Bio pharmacy Medical University of Lublin Chodzki Str. 4, 20-093 Lublin, Poland, [3]Charles E. Schmidt College of Medicine, Florida Atlantic University, Boca Raton, FL

Migraine is a neurological disorder characterized by recurrent and often intense headaches, which can significantly impact individuals, particularly females. The exact mechanisms triggering migraines are not yet fully understood, but scientific evidence suggests that a combination of genetic predisposition and environmental factors play a crucial role in their development. Despite ongoing research, the specific causes of migraines remain a subject of investigation, hindering the availability of definitive treatments for all affected individuals. This study aimed to investigate the neurobiological mechanisms underlying sensory and affective aspects of migraine pain, as well as sociability impairments under a migraine-like condition. The nitroglycerin(NTG) migraine model was employed to mimic migraine-like symptoms by inducing vasodilation through systemic administration. NTG administration resulted in social impairments in both male and female mice. Our research revealed that the administration of a NOP receptor agonist, Ro 64-6198, resulted in a reduction of migraine-like symptoms induced by NTG, such as mechanical allodynia. However, this effect was inhibited when a NOP antagonist, SB-612111, was used. Additionally, in associability study conducted during the migraine condition, Ro 64-6198 demonstrated the ability to alleviate social dysfunctions caused by NTG. Conversely, the effects of the NOP agonist were partially blocked when co-administered with SB-612111. Using the TRAP2/Ai9 mice, our neuroanatomical investigations enabled us to pinpoint the activated neurons and brain regions. We observed that NTG treatment led to a significant increase in activation in various brain regions, including those associated with migraine like pain and social behavior, when compared to animals treated with a vehicle. However, the administration of Ro 64-6198 effectively inhibited the activation of these brain regions induced by NTG, suggesting its potential in mitigating the effects of NTG-induced neuronal hyperactivity. These findings suggest that the activation of NOP receptors modulates neuronal activity in specific brain regions involved in migraine painand social behavior. Our brain-wide analysis will provide us with ample information to better understand the relation between the NOP receptor system and sociability under migraine-like pain.

Key words: Migraine, Pain, Nitroglycerin, NOP, TRAP/Ai9 mice, Sociability impairement

CYP2E1 DNA methylation is associated with altered gene expression in patients with HCV infection

Mateusz Kurzawski , Diana Biniecka , Joanna Łapczuk-Romańska , Marek Droździk
[1]Department of Pharmacology, Pomeranian Medical University in Szczecin, Szczecin, Poland, [2]Department of Pharmacology, Pomeranian Medical University in Szczecin, Szczecin, Poland, [3]Department of Pharmacology, Pomeranian Medical University in Szczecin, Szczecin, Poland, [4]Department of Pharmacology, Pomeranian Medical University in Szczecin, Szczecin, Poland

Introduction. Previous studies have shown that the activity of many drug-metabolizing enzymes is reduced in the course of liver diseases (e.g. hepatitis C, HCV), which may affect the kinetics of drugs taken by patients.
Aim of the study. In the current study we investigated whether the expression of the *CYP2E1* gene in the liver tissue is related to the methylation of the gene's promoter region in patients with liver disease in course of HCV infection.
Material and methods. The study involved HCV patients with various degrees of hepatic insufficiency, according to Child-Pugh score (n=24) and control patients (n=6). Analysis of methylation of 5 cytosines in *CYP2E1* gene promoter in hepatic tissue was performed using the pyrosequencing method. mRNA and protein content was also measured (by real-time PCR and mass spectrometry, respectively).
Results. In HCV patients, the methylation of cytosines 1 and 3 in the gene promoter was significantly higher compared to the liver tissue in the control group. Expression of the *CYP2E1* gene at the mRNA and protein level was lower in HCV patients compared to the control group, and the degree of methylation of the CpG island cytosines showed an inverse correlation with the expression of the CYP2E1 gene, which was statistically significant in the case of cytosine 3.
Conclusions. The results of this study showed that the altered expression of the *CYP2E1* gene in patients with liver disease in the course of HCV infection may be dependent on the cytosine methylation of the *CYP2E1* gene promoter.

Key words: liver disease, drug metabolism, epigenetics

Escitalopram but not venlafaxine reduces an increased RXFP-3 expression in brainstem of adult male Wistar rats exposed to maternal separation

Miłosz Gołyszny [1], Monika Paul-Samojedny [2], Michał Zieliński [1], Ewa Obuchowicz [1]
[1]Department of Pharmacology, Faculty of Medical Sciences in Katowice, Medical University of Silesia, Medyków 18, 40-752 Katowice, Poland, [2]Department of Medical Genetics, Faculty of Pharmaceutical Sciences in Sosnowiec, Medical University of Silesia, Jedności 8, 41-200 Sosnowiec, Poland

Pups and young rats are nursed by the mother rat and it is well-known that maternal care plays an important role in their neurodevelopment. That is why, maternal separation (MS) is commonly used rodent model of early-life stressful events for the study of their long-term consequences that may lead to an increased risk of development of mood and anxiety disorders. There are only a few studies concerning the impact of MS on neuropeptidergic systems. Our previous investigation showed that MS induces significant up-regulation of orexin pathway in the hypothalamus. The current literature suggests that relaxin-3 (RLN-3) system is involved in stress response and pathophysiology of affective disorders. On neurons that express abundantly RLN-3, receptors for stress-dependent hormones are localized.

This study aimed to investigate whether MS and/or escitalopram or venlafaxine given chronically in rats subjected to MS affect RLN-3 system in brain structures of adult male Wistar rats. Similar studies have not been published up to now.
Rats were exposed to MS for 360 min. on postnatal days (PNDs) 2–15. Escitalopram or venlafaxine (10mg/kg) were injected IP once daily from PND 69 to 89. Effects of maternal separation were assessed in behavioral tests (OF and EPM). Peripheral levels of ACTH and corticosterone were measured by immunoenzymatic method. Expression of *RLN-3* and *RXFP-3* (gene of specific receptor for RLN-3) was estimated by quantitative RT-PCR in the following brain structures: brainstem, hypothalamus, amygdala and olfactory bulb. Data were analyzed using one-way ANOVA or Kruskal-Wallis test followed by Student t-test or Mann-Whitney test.
MS induced anxiety-like behavior accompanied by over-activity of the hypothalamic-pituitary-adrenal axis. Expression of *RLN-3* and *RXFP-3* was increased significantly only in the brainstem of MS rats. No significant alterations were detected in the other structures. In the brainstem, escitalopram but not venlafaxine reduced expression of *RXFP-3* up-regulated by MS.
The presented data shed new light on the neurochemical alterations induced by MS because they suggest that relaxin-3 pathway innervating brainstem is involved in the long-term harmful adaptive consequences induced by early-life stress. Chronic administration of escitalopram partly reversed an alteration in RLN-3 system.
This work was supported by a statutory grant (KNW-1-094/N/8/O) from the Medical University of Silesia, Katowice, Poland.

Key words: early-life stress, relaxin-3, RXFP-3, antidepressants

---

Effect of diosmin on changes in the rat skeletal system induced by experimental type 1 diabetes

Joanna Folwarczna [1], Piotr Londzin [1], Weronika Borymska [2], Maria Zych [2], Ilona Kaczmarczyk-Żebrowska [2]
[1]Department of Pharmacology, Faculty of Pharmaceutical Sciences in Sosnowiec, Medical University of Silesia, Katowice, Jagiellońska 4, 41-200 Sosnowiec, Poland, [2]Department of Pharmacognosy and Phytochemistry, Faculty of Pharmaceutical Sciences in Sosnowiec, Medical University of Silesia, Katowice, Jagiellońska 4, 41-200 Sosnowiec, Poland

There is increasing interest in substances of plant origin, which may exert health-promoting activities in diabetes and its complications, including increased fracture risk. Diosmin, a representative of flavonoids, has been reported to exert, among others, antioxidative, antiinflammatory and some antidiabetic effects. Diosmin is widely used in the treatment of venous chronic insufficiency. Taking into account that the diabetic microvascular complications may contribute to bone damage, the aim of the study was to investigate the effects of diosmin on the skeletal system of rats with experimental type 1 diabetes (T1D).
The experiments were carried out on 3-month-old male Wistar rats, divided into four groups: I – healthy control rats, II – streptozotocin-induced diabetic control rats, III –diabetic rats receiving diosmin (50 mg/kg p.o.), IV – diabetic rats receiving diosmin (100 mg/kg p.o.). T1D was induced by a single streptozotocin injection (60 mg/kg i.p.). Diosmin administration, once daily by oral gavage, started two weeks later and lasted four weeks. Serum bone turnover markers, bone mass and mineralization, and mechanical properties of cancellous (the proximal tibial metaphysis) and compact (the femoral diaphysis) were examined.
Diabetes induced profound disorders of bone metabolism and deterioration of cancellous and compact bone strength. Administration of diosmin did not favorably affect the serum bone turnover markers, bone mass and mineralization in the diabetic rats. However, diosmin at a lower dose improved some bone mechanical properties, increasing Young's modulus in the proximal tibial metaphysis (cancellous bone) and the values of the yield point load and energy in the femoral diaphysis (compact bone). There were no significant effects of diosmin at a higher dose on bone mechanical properties.
In conclusion, results of the present study indicate that diosmin may exert some favorable effects on the skeletal system in diabetes.

Key words: diosmin, experimental type 1 diabetes, bone

Effects of sinapic and rosmarinic acids on the skeletal system of ovariectomized rats

Agnieszka Włodarczyk [1], Piotr Londzin [1], Maria Zych [2], Weronika Borymska [2], Ilona Kaczmarczyk-Żebrowska [2], Joanna Folwarczna [1]
[1]Department of Pharmacology, Faculty of Pharmaceutical Sciences in Sosnowiec, Medical University of Silesia, Katowice, Jagiellońska 4, 41-200 Sosnowiec, Poland, [2]Department of Pharmacognosy and Phytochemistry, Faculty of Pharmaceutical Sciences in Sosnowiec, Medical University of Silesia, Katowice, Jagiellońska 4, 41-200 Sosnowiec, Poland

Postmenopausal osteoporosis is the most common type of osteoporosis. It is believed that some polyphenols, including phenolic acids, may counteract the estrogen deficiency-induced bone loss. Oxidative stress, which is associated with aging, plays an important role in the pathogenesis of osteoporosis caused by estrogen deficiency, and phenolic acids are compounds with well-known antioxidant activity. Phenolic acids: sinapic (SA) and rosmarinic (RA), apart of antioxidant activity, were reported to increase serum estradiol concentration in rats. The aim of the study was to investigate the effect of sinapic and rosmarinic acids on the skeletal system of rats with estrogen deficiency induced by bilateral ovariectomy.
The study was carried out on mature female Wistar rats, divided into 7 groups (n=10): SHAM-operated control rats, ovariectomized (OVX) control rats, OVX rats treated with estradiol (0.2 mg/kg; positive control), OVX rats treated with sinapic acid (5 and 25 mg/kg p.o.), and OVX rats treated with rosmarinic acid (10 and 50 mg/kg p.o.). Sham operation or bilateral ovariectomy were conducted 1 week before the start of administration of estradiol or the phenolic acids. The compounds were administered once daily by oral gavage, for 4 weeks. Serum bone turnover markers**,** bone mass, density, mineralization, macrometric and histomorphometric parameters, as well as the mechanical properties were examined.
Estrogen deficiency induced osteoporotic changes in ovariectomized control rats. Intensified bone resorption and formation, worsening of histomorphometric parameters of the femoral metaphysis (bone volume/tissue volume ratio, trabecular separation) and mechanical properties of the proximal tibial metaphysis (cancellous bone) as well as a decrease in bone density were observed. Administration of estradiol slightly favorably affected the skeletal system in OVX rats. The phenolic acids did not counteract changes caused by estrogen deficiency. Moreover, at higher doses, they worsened cancellous bone mechanical properties. In conclusion, the results suggest that the use of sinapic and rosmarinic acids may have a negative impact on the skeletal system in estrogen deficiency.

Key words: sinapic acid, rosmarinic acid, osteoporosis, bone

Effects of Alzheimer's disease and its treatment on the skeletal system

Piotr Londzin, Joanna Folwarczna
Department of Pharmacology, Faculty of Pharmaceutical Sciences in Sosnowiec, Medical University of Silesia, Katowice, Jagiellońska 4, 41-200 Sosnowiec, Poland

There are numerous links between Alzheimer's disease and osteoporosis. Both are diseases of old age that are more common in women than in men. Each disease is considered a risk factor for the other. There is a decreased bone mineral density and an increased risk of bone fractures observed in patients with Alzheimer's disease in relation to the general population. Little is known about the skeletal effects of drugs commonly used in the treatment of Alzheimer's disease, acetylcholinesterase inhibitors (donepezil, rivastigmine, galantamine) and an NMDA receptor antagonist (memantine). There are premises based on scarce epidemiological data that these drugs may exert rather beneficial effects on the skeletal system. Experimental studies on the effects of donepezil

performed so far on mice and rats led to inconsistent results, whereas memantine was reported to favorably affect the skeletal system in a transgenic mice model of Alzheimer's disease under conditions of vitamin D deficiency.
The drugs used in Alzheimer's disease may decrease fracture risk by amelioration of the disease-induced disorders (like cognitive impairment) leading to falls. To dissect the effects of the drugs used in Alzheimer's disease on bones from their other effects, we performed studies concerning the skeletal effects of donepezil and memantine administered for four weeks to healthy rats with normal (non-ovariectomized rats) and reduced estrogen levels (ovariectomized rats; a model of postmenopausal osteoporosis). Both drugs exerted slight unfavorable effects on the skeletal system of non-ovariectomized rats only.
In conclusion, the use of donepezil and memantine may contribute to the adverse skeletal effects observed in Alzheimer's disease. Although donepezil and memantine slightly unfavorably affected the skeletal system of female rats with normal estrogen levels, there was no deleterious effect in conditions of estrogen deficiency. The results of our studies on rats may indicate the relative musculoskeletal safety of donepezil and memantine treatment in postmenopausal women with Alzheimer's disease.

Key words: Alzheimer's disease, skeletal system, donepezil, memantine

---

The effect of tipiracil hydrochloride (TPI), a thymidine phosphorylase (TP) inhibitor, in a rat model of brain ischemia and reperfusion (IR)

Małgorzata Trocha [1], Anna Merwid-Ląd [2], Beata Nowak [2], Tomasz Sozański [2], Adam Szeląg [2], Tomasz Piasecki [3], Paulina Jawień [4], Grzegorz Mazur [5], Adrian Doroszko [5]
[1]Clinical Department of Diabetology and Internal Diseases, Wroclaw Medical University, Borowska 213, 50-556 Wroclaw, Poland, [2]Department of Pharmacology Wroclaw Medical University, Mikulicza-Radeckiego Str. 2, 50-345 Wroclaw, Poland, [3]Department of Epizootiology and Clinic of Bird and Exotic Animals, Wroclaw University of Environmental and Life Sciences, Pl. Grunwaldzki 45, 50-366 Wrocław, Poland, [4]Department of Biostructure and Animal Physiology, Wroclaw University of Environmental and Life Sciences, Norwida 25/27, 50-375 Wroclaw, Poland, [5]Clinical Department of Internal Medicine, Hypertension and Clinical Oncology, Wroclaw Medical University, Borowska 213, 50-556 Wroclaw, Poland

Introduction
Thymidine phosphorylase (TP) is responsible for angiogenesis and apoptosis in cells subjected to IR. It maintains homeostasis, protects neurons, and is also responsible for breaking the blood-brain barrier. TP expression is increased in neurons under IR conditions.
The study aims to evaluate the effect of tipiracil hydrochloride (TPI) – a selective thymidine phosphorylase inhibitor, on the brain tissue subjected to IR in rats.
Material and methods
The study was conducted on 4 groups of Wistar rats. Under general anesthesia, common carotid arteries were isolated and then occluded for 30 min (BCCAO – bilateral common carotid artery occlusion) in groups CIR(n=9), TIR25(n=10), and TIR50(n=9). In group C(n=12), arteries were isolated but not ligated. During ischemia and after 12h of IR, rats received TPI *i.p.* at doses of 25 (TIR25) or 50mg/kg (TIR50). Twenty-four hours after IR, the animals were euthanized. Activities of TP, metalloproteinases (MMP-2, 9), and their inhibitor (TIMP-1) were determined in the serum collected during reperfusion.
Results
After 3h of IR, a significant increase in TP activity in CIR (1.14±0.31ng/ml; **p=0.04)** and TIR25 (1.13±0.2ng/ml; **p=0.03**) in comparison to the C group (0.91±0.22ng/ml) was noticed. In the TIR50 group (1.06±0.17ng/ml), the TP value was not significantly different from the C group at both time points (3 and 24h of IR). There was a non-significant increase in MMP-9 activity in the CIR (4.03±0.62ng/ml) and a decrease in the TIR 25 (3.57±0.52ng/ml). A significant reduction in this group (2.93±0.31ng/ml) compared to the CIR (3.79±0.88ng/ml, **p=0.008**) and TIR 50 (3.88±0.6ng/ml; **p=0.004**) groups was observed after 24 hours of IR. MMP-2 values decreased significantly in all groups (6.45±0.32ng/ml – CIR, 6.41± 0.24ng/ml – TIR25, 6.47±

0.3ng/ml – TIR50) compared to group C (6.95±0.07ng/ml, **p<0.001** in all cases) after 24 h of IR, which correlated with a significant increase in TIMP-1 activity in the TIR25 (10.17±0.6ng/ml; **p=0.025**) and TIR50 (10.34±0.55ng/ml; **p=0.001**) groups compared to C (9.45±0.1 ng/ml) at this time point.
Conclusions
The inhibition of TP activity in the group receiving TPI suggests its protective effect on brain tissue under IR conditions. The increase in MMPs activities associated with IR damage and their decrease in the treated groups suggests a protective effect of TPI on the development of neuroinflammation caused by local brain tissue ischemia.

Key words: tipiracil, thymidine phosphorylase, ischemia-reperfusion, brain tissue, rat

---

Anticonvulsant and antiallodynic activity of new derivatives of pyrrolidin-2,5-dione with a probable novelty mechanism of pharmacological action

Anna Rapacz [1], Kordian Such [2], Dominika Terebus [2], Michał Abram [3], Krzysztof Kamiński [3]
[1]Department of Pharmacodynamics, Faculty of Pharmacy, Jagiellonian University, Medical College, 9 Medyczna St., Kraków, Poland, [2]Faculty of Pharmacy, Jagiellonian University, Medical College, 9 Medyczna St., Kraków, Poland, [3]Department of Medicinal Chemistry, Faculty of Pharmacy, Jagiellonian University, Medical College, 9 Medyczna St., Kraków, Poland

Epilepsy is a chronic neurological disorder that is associated with various types of recurrent seizures, which are drug-resistant in about one third of patients. What's more, anticonvulsant drugs (i.e. pregabalin, gabapentin) are used to treat non-epileptic conditions, including chronic pain, that affects 7–10% of the general population. Thus, there remains a substantial need for the development of more efficacious, better-tolerated treatments for patients with refractory epilepsy and chronic pain.
The aim of the study was to examine anticonvulsant and analgesic activity of new three derivatives of pyrrolidin-2,5-dione: *R*-3, 1 and *R*-1. The experiments were performed using adult male CD-1 mice. Anticonvulsant activity was examined in the maximal electroshock seizure (MES) and psychomotor seizure (6 Hz, 32 mA) tests. The pharmacological studies included also neurotoxicity screening in the rotarod test. Median effective dose (ED_{50}), median toxic dose (TD_{50}) and protective index (PI) were determined. In the next step, the antiallodynic properties were estimated in the model of neuropathic pain caused by oxaliplatin – using the von Frey test. The experimental protocol was approved by the I Local Ethical Committee in Krakow, Poland. The log-probit method was applied to statistically determine the ED_{50} values, and one-way analysis of variance (ANOVA), was used to calculate von Frey test scores.
Anticonvulsant activity demonstrated all tested compounds in the MES test (ED_{50}=38.1–74.2 mg/kg) and in the 6 Hz test (ED_{50}=28.2–43.3 mg/kg). These agents did not impair the motor coordination of animals in rotarod test even at high dose (TD_{50}>200 mg/kg). In the oxaliplatin-induced neuropathy significant antiallodinic activity was observed for both tested compounds *R*-3 (at doses 30, 60 and 90 mg/kg) and *R*-1 (at doses 10, 30 and 60 mg/kg) in the both - first (3 h) and second (7 days) phases.
The results obtained in the current studies proved that in this group of pyrrolidin-2,5-diones, new anticonvulsants with collateral antiallodynic properties can be found. Our recent studies have shown that compounds with a pyrrolidine-2,5-dione moiety revealed a unique and novel mechanism of action, not yet observed in other ASDs, as it is a selective PAM of glutamate transport by EAAT2 (Abram et al. JMedChem 2022).
The study was financially Supported by Funds for Statutory Activity of Jagiellonian University Medical College, Kraków, Poland (N42/DBS/000243 and N42/DBS/000328).

Key words: epilepsy, seizure, neuropathic pain

The effect of verbal suggestion on the response generalization of the nocebo effect. A pilot study.

Marta Jakubińska, Jacek Skalski, Wacław M. Adamczyk
Laboratory of Pain Research, Academy of Physical Education in Katowice, Katowice, Poland

Background and aims
Although research clearly indicates that appropriately formulated verbal suggestion can increase symptoms' sensations (nocebo effect), it is unclear whether this effect is limited to the symptom to which the suggestion was applied or extends to other similar symptoms. This experiment aimed to answer the question whether inducing nocebo hyperalgesia through verbal suggestion may increase the sensation of another symptom (i.e., paresthesia).
Methods
Ten volunteers participated in the study, randomly allocated to one of two groups: control (n=5) and nocebo (n=5). Compression stimuli were applied using a blood pressure cuff, inducing two symptoms simultaneously: pain and paraesthesia. Participants rated the level of symptoms experienced in real-time using coVAS sliders. The experiment consisted of two parts: "pretest" and "posttest," with identical pressure parameters and a 10-minute break between. In the nocebo group, verbal suggestion was used to suggest an increase in compression strength.
Results
In the control group, there were no significant differences between pretest and posttest for pain ($p=0.23$) or paresthesia ($p=0.14$). In the experimental group, a statistically significant nocebo effect for pain was observed ($p=0.04$). The groups did not differ in symptom severity at pretest ($p=0.35$).
Conclusions
The results suggest that the verbal suggestion may induce a nocebo hyperalgesia effect, but it did not generalize to another symptom. Although a trend towards generalization was observed, further research with a larger sample size is necessary to answer the question of whether response generalization of the nocebo effect is possible using verbal suggestion.