ABSTRACT BOOK

2nd Collection of Seminars of PhD. Students

2024

Center of Experimental Medicine
Slovak Academy of Sciences

"Health blooms in the garden of research,
pharmacology tends to its growth,
and toxicology prunes away danger."

03/2023 - 04/2024

2nd Collection of Seminars of PhD. Students
Centre of Experimental Medicine
Slovak Academy of Sciences
ABSTRACT BOOK

Organized by

Group of PhD Students and Young Scientific Workers of the Center Of Experimental Medicine of the Slovak Academy Of Sciences

Centre of Experimental Medicine, Slovak Academy of Sciences
Dúbravská cesta 9, 841 04 Bratislava, Slovakia

Institute of Experimental Pharmacology & Toxicology
Institute for Hearth Research
Institute of Normal and Pathological Physiology

Electronic reviewed Abstract book

ISBN 978-80-89991-14-3

Registered by the Ministry of Culture of the Slovak Republic

TABLE OF CONTENTS

COMMITTEES

Organising Committee

- **Ing. Peter Pôbiš**
  Institute of Experimental Pharmacology & Toxicology, CEM SAS
- **Ing. Marek Lepáček**
  Institute of Experimental Pharmacology & Toxicology, CEM SAS
- **Mgr. Kristína Ferenczyová, PhD.**
  Institute for Hearth Research, CEM SAS
- **Ing. Viktoria Pecniková**
  Institute for Hearth Research, CEM SAS
- **Mgr. Michal Kluknavský, PhD.**
  Institute of Normal and Pathological Physiology, CEM SAS

Abstract book editors

- **Ing. Peter Pôbiš**
- **Ing. Marek Lepáček**

Abstract book reviewers

- **Ing. Filip Kvetoň, PhD.**
  Institute of Chemistry, SAS
- **Ing. Zoltán Polozsányi, PhD.**
  Institute of Biochemistry and Microbiology, FCFT SUT
- **Ing. Lucia Pažitná, PhD.**
  Institute of Chemistry, SAS

ISBN 978-80-89991-14-3 *(online version)*

Registered by the Ministry of Culture of the Slovak Republic

ORAL PRESENTATIONS

03/2023 | Drug-gut microbiota interaction of novel aldose reductase inhibitor cemtirestat in ZDF rats.

Marek Lepáček, Pavol Boďo, Milan Štefek, Marta Š. Prnová

Centre of Experimental Medicine, Institute of Experimental Pharmacology & Toxicology, Slovak Academy of Sciences, Bratislava, Slovakia

The human gut microbiome is a complex ecosystem, that mediates host interactions with its environment (Weersma, 2020). Individual humans are up to 99.9% genetically identical to each other in terms of their host genome, while compared to the gut microbiota, humans can differ from each other by 80-90%. This suggests the uniqueness and importance of the gut microbiome (Ursell, 2012). In addition, the gut microbiome can metabolize drugs, and drugs can change the composition of the microbiome. Currently, these interactions are still relatively poorly described and unclear (Flowers, 2020). For example, the anti-diabetic drugs metformin and thiazolidinediones can change the composition and function of the microbiome (Whang, 2019).
Our study focuses on the new multitarget drug in the therapy of secondary diabetic complications. Cemtirestat is characterized as a highly selective and effective inhibitor of aldose reductases, the first enzyme of the polyol pathway. In addition, multiple antioxidant abilities were observed [5]. In the present study, we focused on 3-month-old "Zucker diabetic fatty (ZDF) "lean" rats treated with cemtirestat in a dosage of 7.7 mg/kg/day for 2 months. The biochemical analysis of blood was performed. The intestinal microbiota composition was determined using a whole genome sequencing method of rat fecal samples. ZDF "lean" rats showed a favorable effect on the amount of microflora compared to the control group.
Biochemical analyses including 20 parameters were performed. The ratio Firmicutes/Bacteroidetes, (F/B) was without significant change in rats feaces treated with cemtirestat. The Shanon index of alpha diversity didn´t show any significant shift compared to the control group, as well as the main phyla abundance. Furthermore, analysis of Clostridium butyricum and Lactobacillaceae promotes the stimulating effect of cemtirestat on the abundance of the mentioned species. We also observed a decrease in Clostridium difficile species in rats treated with cemtirestat.
Our study suggests that cemtirestat has no damaging impact on the rat's gut microbiome. In addition, cemtirestat shows a stimulatory effect on some species of probiotic bacteria, the decline of which is closely related to the progression of T2DM.

Acknowledgment

This contribution was created with the support of the Research and Development Agency under project number APVV-20-0411sa VEGA-2/0087/22.

References

R. K. Weersma, A. Zhernakova, a J. Fu, “Interaction between drugs and the gut microbiome”, Gut, roč. 69, č. 8. BMJ, Publishing Group, s. 1510–1519, aug. 01, 2020. doi: 10.1136/gutjnl-2019-320204.

L. K. Ursell, J. L. Metcalf, L. W. Parfrey, a R. Knight, “Defining the Human Microbiome”, Nutr Rev, roč. 70, č. Suppl 1, s. S38, aug. 2012, doi: 10.1111/J.1753-4887.2012.00493.X.

S. A. Flowers, S. Bhat, a J. C. Lee, “Potential Implications of Gut Microbiota in Drug Pharmacokinetics and Bioavailability”, Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy, roč. 40, č. 7, s. 704–712, júl. 2020, doi: 10.1002/PHAR.2428.

A. Whang, R. Nagpal, a H. Yadav, “Bi-directional drug-microbiome interactions of anti-diabetics”, EBioMedicine, roč. 39, s. 591–602, jan. 2019, doi: 10.1016/J.EBIOM.2018.11.046.

04/2023 | Neurocognitive Mechanisms of Semantic Memory.

Adam Kubinec, Martin Marko

Centre of Experimental Medicine, Institute of Normal and Pathological Physiology, Slovak Academy of Sciences, Bratislava, Slovakia

Semantic memory is important for encoding, storing, and recalling knowledge, and thus for forming concepts and understanding language. Effective handling of information from semantic memory is crucial for the ability to use, manipulate, and generalize knowledge that people acquire throughout their lives (Kumar, 2021). The current model assumes that semantic cognition consists of two interacting systems: a semantic representation system and a semantic control system, such a model is also referred to as the Controlled Semantic Cognition framework. Semantic representation encodes conceptual representations of words and concepts, by learning relationships between different information already stored in semantic memory. The second system is semantic control, which executively regulates activation in the representational system to generate inferences and behaviors that are appropriate for the current situation or task (Jefferies et al., 2020; Ralph et al., 2017).
The main goal of our research is to validate and extend current perspectives on semantic cognition (e.g., Jefferies et al., 2020; Ralph et al., 2017), exploring and describing the underlying neurocognitive mechanisms that underlie the ability to retrieve, executive control, and access conceptual representations. We will examine the functional role of prefrontal and temporal cortical brain regions in semantic processes using non-invasive brain electrical stimulation (tES). We will also focus on the modulation of relevant neurobiological systems that underlie semantic memory information processing and its control (e.g., the role of the central noradrenergic system, and stress hormones).

References

Jefferies, E., Thompson, H., Cornelissen, P., & Smallwood, J. (2020). The neurocognitive basis of knowledge about object identity and events: dissociations reflect opposing effects of semantic coherence and control. Philosophical Transactions of the Royal Society B, 375(1791). https://doi.org/10.1098/RSTB.2019.0300

Kumar, A. A. (2021). Semantic memory: A review of methods, models, and current challenges. Psychonomic Bulletin & Review, 28(1), 40–80. https://doi.org/10.3758/s13423-020-01792-x

Ralph, M. A. L., Jefferies, E., Patterson, K., & Rogers, T. T. (2017). The neural and computational bases of semantic cognition. https://doi.org/10.1038/nrn.2016.150

09/2023 | The effect of Nelumbo nucifera leaves on rats with adjuvant arthritis

Katarína Pružinská[1,2], Martin Chrastina[1,2], Sasan Khademnematolahi[1,3], Silvester Poništ[1], František Dráfi[1], Pham Ngoc Khanh[4], Katarína Bauerová[1]

[1]Centre of Experimental Medicine, Institute of Experimental Pharmacology & Toxicology, Slovak Academy of Sciences, Bratislava, Slovakia

[2]Comenius University in Bratislava, Jessenius Faculty of Medicine in Martin, Department of Pharmacology, Martin

[3]Comenius University in Bratislava, Faculty of Natural Sciences, Bratislava

[4]Vietnam Academy of Science and Technology, Institute of Natural Products Chemistry, Hanoi, Vietnam

Nelumbo nucifera – more commonly known as sacred lotus, contains active phytochemicals (mainly flavonoids, alkaloids, polysaccharides, polyphenols, and essential oils) responsible for various biological or pharmaceutical activities – antioxidant activity, modulation of immune function, hypoglycaemic activity, and anti-obesity activity. The supplementation could potentially alleviate the symptoms of rheumatoid arthritis through its influence on pathological inflammatory processes.
In our experiment, we aimed to select a suitable application form (alcoholic extract or dry powder from Nelumbo nucifera leaves and their suitable dose) and then assess the effect of the selected dose in monotherapy and in combined therapy with methotrexate on the observed biometric parameters (weight change, hind paw volume, arthrogram) and biochemical parameters (Il-1β, IL-17A, and MMP-9) in Lewis rats with adjuvant arthritis.
Based on the preliminary study, the alcoholic extract, and the highest dosage of N. nucifera exerted better clinical results. In the following experiment, the monotherapy and combinational therapy significantly modified clinical parameters: change of animal weight, change of hind paw volume, and arthrogram. In addition, the combination therapy of MTX and N. nucifera was more effective than MTX alone in the reduction of swelling of the hind paw observed on all experimental days. Biochemical analysis showed no significant differences in monotherapy in reducing levels of Il-1β, IL-17A, and MMP-9 in plasma samples, however the combinational therapy of MTX and N. nucifera more effectively reduced levels of IL-17A in plasma on 14th and 28th day than MTX alone.
These results are the basis for further investigation of inflammatory parameters in plasma and relevant tissues, such as cytokines and antioxidant enzymes.

Acknowledgment
VEGA 2/0136/20, VEGA 2/0126/20, VEGA 2/0091/20, VAST-SAS bilateral project QRSK01.03/21/22 “Anti-inflammatory effects of natural compounds isolated from Vietnam medicinal plants“.

09/2023 | The relationship between genetic variability of brain-derived neurotrophic factor and memory consolidation during sleep in humans

Rastislav Rovný

Centre of Experimental Medicine, Institute of Normal and Pathological Physiology, Slovak Academy of Sciences, Bratislava, Slovakia

The brain-derived neurotrophic factor (BDNF) is an essential regulator of synaptic plasticity, a candidate neurobiological mechanism underlying learning and memory. A functional polymorphism in the BDNF gene, Val66Met (rs6265), has been linked to memory and cognition in healthy individuals and clinical populations. Sleep contributes to memory consolidation, yet information about the possible role of BDNF in this process is scarce. To address this question, we investigated the relationship between the BDNF Val66Met genotype and consolidation of episodic declarative and procedural (motor) non-declarative memories in healthy adults. The carriers of the Met66 allele, as compared with Val66 homozygotes, showed stronger forgetting overnight (24 h after encoding), but not over a shorter time (immediately or 20 min after word list presentation). There was no effect of the Val66Met genotype on motor learning. These data suggest that BDNF plays a role in neuroplasticity underlying episodic memory consolidation during sleep.

10/2023 | Exploring treatment options in the therapy of animal model of pharmacoresistant depression.

Alexandra Ballóová[1,2], Stanislava Bukatová[1], Mojmír Mach[1]

[1]Centre of Experimental Medicine, Institute of Experimental Pharmacology & Toxicology, Slovak Academy of Sciences, Bratislava, Slovakia

[2]Department of Pharmacology, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Slovakia

Major depressive disorder is a serious multifactorial illness that affects nearly 280 million people globally. Despite the wide availability of antidepressants, up to 30% of patients exhibit poor response to initial treatment, falling into the category of pharmacoresistant depression (FRD). This emphasizes the imperative need for the exploration of novel therapeutics. In a previous study, we unveiled the antidepressant effects of the new pyridoindole derivative SMe1EC2M3, a potential triple reuptake inhibitor, following acute administration [1]. In our ongoing study, we turned our attention to another promising compound, Zoletil®, combining the N-methyl-D-aspartate receptor antagonist tiletamine with the benzodiazepine zolazepam, co-administered with venlafaxine for FRD treatment.

In our experimental study, we explored the impact of chronic oral administration of SMe1EC2M3 (dose 10mg/kg) and the combination of Zoletil® (10mg/kg in the 1st week of treatment) with venlafaxine (Alventa 150mg, 10mg/kg in the 2nd, 3rd, and 4th weeks of treatment) under conditions of stress induced by a 4-week chronic mild stress procedure in Wistar-Kyoto male rats. We assessed depressive-like and anxiety-like behavior, and spontaneous locomotion using the sucrose preference test (SPT), open field test (OF), elevated plus maze test (EPM), and forced swim test (FST). We also evaluated cognition, particularly recognition memory in the novel object recognition test (NOR). Behavioral data were analyzed using one-way ANOVA and Fisher LSD post-hoc tests.

Sucrose preference was significantly reduced in the venlafaxine group, acting as a negative control, compared to the control. The SMe1EC2M3 group exhibited increased sucrose consumption compared to the venlafaxine group. In the OF, there were no significant changes in the total distance traveled. However, we observed a significantly higher number of entries into both the central and peripheral zones in the venlafaxine and SMe1EC2M3 groups compared to the Zoletil® + venlafaxine group. In EPM we observed a significant difference in time spent in open arms of the maze in the venlafaxine and Zoletil® group, which might open the question about the anxiolytic effect of Zoletil®. In the time spent in closed arms, we see a significant difference in the SMe1Ec2M3 group in comparison with controls. In FST we observed two parameters: in time spent floating we did not observe any significant differences between groups. However, in time spent climbing we see a significant effect of pyridoindole derivative in comparison with the control and venlafaxine group. The NOR test revealed a significantly higher discrimination ratio in the SMe1EC2M3 group compared to all other groups, indicating that rats explored the new object more frequently than the familiar one.

Our findings suggest that SMe1EC2M3 might have the potential to ameliorate some behavioral changes associated with FRD. Additionally, the results indicate that initial treatment with Zoletil® then switched to venlafaxine seems to have an anxiolytic effect rather than an antidepressant.

References

[1] Koprdova, R. et al. Electrophysiology and Behavioral Assessment of the New Molecule SMe1EC2M3 as a Representative of the Future Class of Triple Reuptake Inhibitors. Molecules, 2019; 24(23):4218. Supported by the grant VEGA- 2/0154/20.

10/2023 | ACE2 inhibition induces oxidative damage and upregulated gene expression of antioxidants in the brainstem of spontaneously Hypertensive Rats.

Michal Kluknavský, Andrea Mičurová, Martina Cebová, Ezgi Şaman, Soňa Čačányiová, Iveta Bernátová

Centre of Experimental Medicine, Institute of Normal and Pathological Physiology, Slovak Academy of Sciences, Bratislava, Slovakia

Angiotensin 1–7 is a peptide produced by the angiotensin-converting enzyme 2 (ACE2). The SARS-CoV-2 virus can bind to the ACE2, which leads to the virus entering cells and blocking ACE2 activity. Decreased bioavailability of angiotensin 1–7 due to ACE2 blockade may contribute to increased mortality in hypertensive individuals during COVID-19 disease. However, the effects of ACE2 inhibitor MLN-4760 on brain function remain unknown.

We investigated selected behavioral and hemodynamic parameters in spontaneously hypertensive rats (SHR) after a 2-week s.c. infusion of MLN-4760 (dose 1 mg/kg/day). The biochemical and molecular effects of MLN-4760 were investigated in the brainstem (BS) and blood plasma. Systolic blood pressure and heart rate were determined by tail-cuff plethysmography. Exploratory and anxiety-like behaviors were investigated by an open-field test. The total activity of nitric oxide synthase (NOS) was determined by [3H]-L-citrulline formation from [3H]-L-arginine. Gene expressions were studied by the two-step RT-qPCR method. Hydrogen sulfide (H2S) concentration in plasma was measured via methylene blue assay. We determined the conjugated dienes (CD) content as a marker of oxidative damage.

MLN-4760 had no effects on hemodynamic and behavioral parameters. However, MLN-4760 increased plasma H2S level, NOS activity, and CD level in the BS. Increased NOS activity correlated positively with gene expression of Nos3 while plasma H2S levels correlated positively with gene expressions of H2S-producing enzymes Mpst, Cth, and Cbs. MLN-4760 treatment increased gene expression of Ace2, Sod1, Sod2, Gpx4, and Hmox1, which positively correlated with the expression of gene Nfe2l2 encoding the transcription factor NRF2.

Collectively, MLN-4760 did not exacerbate pre-existing hypertension and behavioral hyperactivity/anxiety in SHR. However, MLN-4760-induced oxidative damage in BS was associated with activation of NO- and H2S-mediated compensatory mechanisms and increased gene expression of antioxidant, NO- and H2S-producing enzymes correlated positively with elevated Nfe2l2 expression.

Acknowledgment

Study supported by the grants SRDA No. PP-COVID-20-0043 and VEGA No. 2/0157/21.

11/2023 | Plasma oxidative markers and cardiac ischemia-reperfusion damage in hypertensive SHR rats following long-term flavonoid quercetin treatment

Ulrika Dulova[1], Kristina Ferenczyova[1], Lucia Kindernay[1], Jana Vlkovicova[1], Lubomira Tothova[2], Monika Bartekova[1,3]

[1]Centre of Experimental Medicine, Institute for Heart Research, Slovak Academy of Sciences, Bratislava, Slovakia

[2]Institute of Molecular Biomedicine, Faculty of Medicine, Comenius University, Bratislava, Slovakia

[3]Institute of Physiology, Faculty of Medicine, Comenius University, Bratislava, Slovakia

Introduction: It has been demonstrated that the naturally occurring polyphenol quercetin (QCT) provides cardioprotective and antioxidant characteristics. Studies suggest that QCT protect the rat heart from ischemia-reperfusion (I/R) damage when comorbidities are absent. We used a spontaneously hypertensive rats (SHR) to test whether QCT can protect the heart against I/R even in the presence of comorbidities, specifically hypertension, given the incidence of cardiac I/R injury, such as myocardial infarction, in patients with various comorbidities. QCT was additionally analyzed for plasma oxidation levels and blood pressure.

Methods: Six weeks of oral QCT (20 mg/kg/day) has been administered to adult 3-month-old SHR rats. Measurements of blood pressure were taken before and after QCT was given. Following the sacrifice of the animals, I/R (30/120 min) was produced in isolated hearts using the Langendorff perfusion technique. At the conclusion of I/R, the infarct size was measured. The analysis of plasma indicators of oxidative stress (AOPP, AGEs, FRAP, and TBARS) was done using the appropriate kits.

Results: Using QCT allowed us to see a trend toward lessening the increase in left ventricular end-diastolic pressure as well as a positive trend in the recovery of cardiac function after I/R. After ischemia, all hearts with QCT returned to normal, but 22% of hearts lacking QCT never recovered and entered a persistent fibrillation. Both infarct size and plasma oxidative stress indicators were unaffected by QCT. Lastly, the rats' blood pressure was unaffected by QCT.

Conclusion: QCT appears to be a potential treatment for preventing I/R harm in hypertensive individuals. It mostly improves the heart's electrical and mechanical performance after I/R, but it does not reduce the size of the infarct. This cardioprotective benefit in hypertensive individuals is not likely to be attributable to QCT's systemic antioxidant action.

Acknowledgment
Supported by APVV-21-0194 and VEGA 2/0104/20 and 2/0159/24

11/2023 | Oxidative damage mitigation in the heart after irradiation

Patricia Pavelkova, Barbora Kalocayova, Jan Slezak, Branislav Kura

Centre of Experimental Medicine, Institute for Heart Research, Slovak Academy of Sciences, Bratislava, Slovakia

The overproduction of free radicals is a key factor in the development of numerous diseases, including cardiovascular conditions. A common source of oxidative stress is radiation exposure, particularly in medical settings such as radiotherapy and X-ray imaging. This irradiation and subsequent oxidative stress can cause damage of healthy cells and tissues by its effects on biomacromolecules or by water radiolysis. Many studies suggest that molecular hydrogen (H2) can help mitigate oxidative damage.
In our study, we used 3-months-old Wistar rats that underwent a single dose of 10 Gy irradiation in the mediastinum area. The animals were divided into two experimental groups, one receiving H2 by inhalation of hydrogen-air mixture (4% H2, 3x30min) and the other one drinking hydrogen-rich water (3x3 mL, 2 ppm). The samples were collected two and nine days after irradiation.
We detected the decrease in measured parameters of oxidative stress (malondialdehyde, superoxide, glutathione peroxidase, superoxide dismutase), inflammatory parameters (NFκB) in both experimental groups treated with H2 compared to untreated animals.
Our results suggest that H2 could be an effective strategy for treating heart damage caused by irradiation. In our experimental model, H2 administration via inhalation appears to be more efficient. These findings provide a foundation for further investigation into inflammatory parameters in plasma and relevant tissues, including cytokines and antioxidant enzymes.

Acknowledgment
This work was supported by grants VEGA (2/0063/18, 2/0092/22, and 2/0148/22), APVV (APVV-15-0376, APVV-19-0317) and grant of The Ministry of Education, Science, Research and Sport of the Slovak Republic (2019/4-CEMSAV-1).

01/2024 | Effect of dimethyl fumarate on the expression of NRF2 target genes in hypertriglyceridemic rats

Aybüke Bozkurt, Iveta Bernátová

Centre of Experimental Medicine, Institute of Normal and Pathological Physiology, Slovak Academy of Sciences, Bratislava, Slovakia

The rising prevalence of metabolic disorders like obesity and hyperlipidemia is associated with an increased risk for the development of coronary artery disease, hypertension, and heart failure. From the literature, nuclear factor erythroid 2-related factor 2 (NRF2) expression is downregulated in hyperlipidemia, hypertension, and inflammation[1]. Activation of NRF2 by using pharmacological or natural products is a promising therapeutic approach for the treatment of obesity and cardiovascular diseases. NRF2 is a transcription factor that regulates the cellular defense against toxic and oxidative insults by expressing genes involved in antioxidant defense and drug detoxification2. In addition to antioxidant responses, NRF2 is involved in many other cellular processes, including lipid metabolism and cell inflammatory responses[1,2]. Dimethyl fumarate (DMF) is known for its NRF2-activating properties. By modulating the expression of NRF2 target genes, DMF should enhance the cellular defense mechanisms against these harmful processes, making it a potential treatment option for conditions involving oxidative stress, inflammation, and lipid metabolism. By modulating NRF2, DMF may help restore redox balance, reduce inflammation, and improve vascular function[3].

This study investigates how DMF will affect parameters such as blood pressure (BP), oxidative stress, and expression of NRF2 and NRF2-regulated genes in hereditary hypertriglyceridemic (HTG) rats with elevated BP. The study will also use Wistar-Kyoto (WKY) rats as normotensive control groups without metabolic disorders. The hypothesis is that DMF treatment reduces BP in WKY and HTG as well as improves functional parameters of prehypertension and/or hypertriglyceridemia and changes the expression patterns of NRF2 target genes in selected tissues.

References

Gutiérrez-Cuevas J, Galicia-Moreno M, Monroy-Ramírez HC, Sandoval-Rodriguez A, García-Bañuelos J, Santos A, Armendariz-Borunda J. The Role of NRF2 in Obesity-Associated Cardiovascular Risk Factors. Antioxidants (Basel). 2022 Jan 26;11(2):235. doi: 10.3390/antiox11020235. PMID: 35204118; PMCID: PMC8868420.

He F, Ru X, Wen T. NRF2, a Transcription Factor for Stress Response and Beyond. Int J Mol Sci. 2020 Jul 6;21(13):4777. doi: 10.3390/ijms21134777. PMID: 32640524; PMCID: PMC7369905.

Lastres-Becker I, García-Yagüe AJ, Scannevin RH, Casarejos MJ, Kügler S, Rábano A, Cuadrado A. Repurposing the NRF2 Activator Dimethyl Fumarate as Therapy Against Synucleinopathy in Parkinson's Disease. Antioxid Redox Signal. 2016 Jul 10;25(2):61-77. doi: 10.1089/ars.2015.6549. Epub 2016 Apr 27. PMID: 27009601; PMCID: PMC4943471

01/2024 | Effects of mesenchymal stem cells and HMGB1 inhibitor on the cardiovascular system after experimentally induced myocardial infarction in hypertension

Katarína Bujnová, Martina Cebová

Centre of Experimental Medicine, Institute of Normal and Pathological Physiology, Slovak Academy of Sciences, Bratislava, Slovakia

Cardiovascular diseases, including myocardial infarction, are the most common cause of death worldwide. Myocardial infarction is a form of ischemic heart disease that arises mainly from atherosclerosis. Severe ischemia results in left ventricular remodeling, characterized by progressive replacement of the surviving contractile tissue by fibrosis, cardiac tissue damage, and heart failure from the large loss of functional cardiomyocytes. Hypertension is one important risk factor for myocardial infarction.
The myocardium itself does not have a high regenerative capacity, and it is important to minimize the loss of cardiomyocytes and maintain cardiac function after myocardial infarction. Mesenchymal stem cell (MSC)-based therapy has emerged as a novel alternative treatment for MI. Administration of MSCs can attenuate cardiac remodeling and improve heart function recovery following MI by inhibiting cardiomyocyte apoptosis and inflammation, increasing angiogenesis, and rejuvenating cardiac muscle cells, as well as increasing vascular density and improving myocardial structure and function after MI.
High mobility group box 1 (HMGB1) protein is a nuclear, chromatin-binding protein that regulates transcription, DNA replication, and DNA repair. In the extracellular space, HMGB1 acts as a damage-associated molecular pattern (DAMP) involved in a large variety of different processes, such as inflammation, migration, invasion, proliferation, differentiation, and tissue regeneration. It is also released by damaged cardiomyocytes after myocardial infarction. It acts via toll-like receptors and activates the production of proinflammatory cytokines such as tumor necrosis factor alpha or interleukin 6 by nuclear factor kappa B activation.
The aim of our study is to clarify the importance of nitric oxide in myocardial infarction and to define early molecular and morphological changes that are caused by either stem cell application or glycyrrhizin, HMGB1 inhibitor, administration after myocardial infarction in hypertensive conditions.

03/2024 | The cardioprotective effect of remote ischemic preconditioning and protective signaling pathways in aging rats

Lucia Kindernay, Monika Barteková

Centre of Experimental Medicine, Institute for Heart Research, Slovak Academy of Sciences, Bratislava, Slovakia

Cardiovascular diseases are among the most serious and most widespread diseases. Mortality due to cardiovascular diseases represents 50% of total mortality in the world, but most in developed countries. Ischemic heart disease participates to this mortality over a half and can be affected by several risk factors and diseases, such as hyperlipidemia, hypertension, diabetes mellitus, or aging. Age itself is a very significant risk factor and may cause molecular, structural, or biochemical changes in the whole cardiovascular system.

The phenomenon known as ischemic preconditioning (IPC) was first described in 1986 by Murry et al. It is an adaptive response in which brief exposure to ischemia/reperfusion (I/R) markedly enhances the ability of the heart to withstand a subsequent sustained ischemic injury. Whether cardioprotection by IPC is changed in aged myocardium has been studied in animal models as well as in the human heart. However, the results on the effectiveness of IPC in the aged myocardium remain controversial. Most studies in animal models prove, that increased age may cause loss of cardioprotection by IPC. However, some of these studies demonstrate the preservation of cardioprotection even in elderly animals (12 months) but not in very old animals (24 months).

Classical ischemic preconditioning is a very effective form of adaptation, however, it is difficult to use in a clinical condition, because it requires an invasive intervention directly into the myocardium to achieve its cardioprotective effect. An alternative and more accessible strategy is the application of a cardioprotective stimulus in an organ or tissue distant from the heart. This phenomenon, when even a short episode of ischemia induced in other tissues or organs mediates an increase in the heart's resistance to longer-lasting ischemia, is called remote ischemic preconditioning (RIPC). It was first discovered by Przyklenk et al. in 1993 and since then it has become one of the most actively studied forms of cardioprotection. In many studies, a positive effect of RIPC has already been found in both young and elderly patients (used before congenital cardiac defects surgery, coronary artery bypass grafting, etc.). However, little is known about the effect of RIPC and its molecular basis in elderly animals.

Our work focuses on clarifying the effect of RIPC on the resistance of the heart against I/R injury and identifying proteins involved in protective pathways in aging 13 months old Wistar rats. Langendorff-perfused hearts were exposed to 30-min ischemia/120-min reperfusion without or with prior RIPC. RIPC consist of 3 cycles, 5-min ischemia /5-min reperfusion, and was applied on the hind limb of anesthetized rats (pressure cuff inflation - 200 mmHg/deflation - 0 mmHg). We measured infarct size (IS), susceptibility to ventricular arrhythmias, and recovery of contractile function (left ventricular developed pressure - LVDP). In parallel groups, left ventricular tissue was sampled for the detection of protein levels of RISK (pAkt/Akt, p-eNOS/eNOS, PKCε, pGSK3β/GSK3β, pERK/ERK, JNK2), SAFE (pSTAT3/STAT3, JAK1) and pro/anti-apoptotic pathways (Bax/Bcl-2, Casp-3).

Acknowledgment
Supported by projects APVV-19-0540, VEGA 2/0141/18, APVV-21-0194.

03/2024 | Purpose, knowledge, and benefits of a scientific stay at the Cardiovascular Institute in the Center for Biomedical Research and Translational Surgery at the Medical University of Vienna completed in autumn 2023

Kristína Ferenczyová, Monika Barteková

Centre of Experimental Medicine, Institute for Heart Research, Slovak Academy of Sciences, Bratislava, Slovakia

In recent decades, significant progress has been made in the research of mechanisms involved in cardioprotection, which in turn contributed to the discovery of several cardioprotective pathways and interventions. However, the demand to develop new tools for cardioprotection is still highly relevant, as the prevalence of serious cardiovascular diseases and heart failure continues to grow globally.

However, the difficulty of this research represents a great challenge from several points of view, namely infrastructure, laboratory equipment, research funding, and human resources. One of the ways to contribute to the development of research, even if one of the mentioned factors is an obstacle, is to try to establish cooperation with a workplace of a similar focus. In addition, in the scientific environment, the creation of national or international collaborations is highly valued, as evidenced by the great support of grant agencies.

For the sake of development and progress on the scientific level, the potential of mutual cooperation was discussed by the group led by Doc. Barteková in the Institute of Heart Research CEM SAS and the group of Dr. Attila Kiss with a similar scientific focus on cardioprotection, who carries out his work at the Cardiovascular Institute in the Center for Biomedical Research and Translational Surgery at the Medical University of Vienna under the supervision of director Prof. Bruno Podesser. The real start of this collaboration was my stay at a partner workplace, for which I received a scholarship financed by the Austrian agency OeAD for the support of education and internationalization for a 3-month stay, which took place between September 1 and November 30, 2023. One of the purposes of this stay, in addition to starting active cooperation, was to learn new methodologies with the goal of implementing these methods at our workplace. Another purpose was to propose an experimental design that will form an intersection of the interests of both workplaces. Last but not least, a significant part of my stay was dedicated to finding a suitable bilateral grant scheme and submitting a joint project. Therefore, the output of the stay was not only for a short-term or just a personal interest, but it also brings the potential for the creation of good relations between both workplaces and opens the possibility for strengthening scientific cooperation. Moreover, this particular possibility of long-term maintenance of this cooperation is supported due to the short distance and excellent accessibility between both workplaces.

Based on the above mentioned, I would like to use the opportunity of the periodically organized seminar to present an insight into what I´ve learned and experienced during this valuable stay and support the idea of traveling for its great value on a personal and professional level.

04/2024 | Utilisation of In Vitro 3D Reconstructed Tissue Models for Assessing Drug Candidates Toxicity in COVID-19 Therapies

Peter Pôbiš[1], Helena Kanďárová[1,2]

[1]*Centre of Experimental Medicine, Institute of Experimental Pharmacology & Toxicology, Slovak Academy of Sciences, Bratislava, Slovakia*

[2]*Faculty of Chemical and Food Technology, Institute of Biochemistry and Microbiology, Slovak University of Technology in Bratislava, Bratislava, Slovakia*

The event of the SARS-CoV-2 pandemic has shown the urgent need for effective therapeutic interventions. The pandemic resulted in a global race to identify molecules with potential treatment effects. However, the safety of the drug candidates needs to be our priority. Traditional preclinical toxicity assessments often rely on animal models, which may not fully recapitulate human physiology and present ethical concerns. In this context, leveraging in vitro 3D reconstructed tissue models offers a promising alternative for evaluating drug toxicity with greater relevance to human biology.

At the Centre of Experimental Medicine SAS, we addressed two projects centered on combating COVID-19. In the framework of these projects, several materials were identified, that manifested promising physicochemical properties that could potentially have therapeutic effects against the virus and its associated complications. One of our key objectives was to evaluate the toxicity of these materials.

To evaluate basic cytotoxicity, we employed the VERO E6 cell line following the ISO 10993-5 guidelines, utilizing the MTT test. In addition, we conducted in vitro evaluations of inhalation toxicity using the reconstructed 3D human lung model EpiAirway, and intestinal toxicity using the reconstructed 3D human small intestine model EpiIntestinal. These models were selected based on the assumption, that these potential drugs could be administered orally or by inhalation. The inhalation toxicity evaluation on EpiAirway was conducted using VITROCELL® Cloud Alpha MAX, which allowed us to simulate a real-life scenario, of inhaling the aerosol containing drug, via nebulization. In both 3D models, EpiAirway and EpiIntestinal, several endpoints were evaluated, encompassing transepithelial electrical resistance (TEER) measurement, viability assessment through the MTT assay, and cytokine analysis.

Remarkably, both 3D tissue models demonstrated remarkable resilience against higher concentrations of the tested materials compared to simple cell cultures, suggesting their potential for subsequent antiviral activity testing. This enhanced specificity can be attributed to the models' robust barrier properties and mucus-generation capacity.

Moving forward, we aim to collaborate with external experts to explore the antiviral and antimicrobial potential of these materials, focusing on concentrations known for their biological compatibility. Our study emphasizes the importance of employing in vitro methods to uncover acute inhalation and intestinal toxicity characteristics associated with promising bioactive agents.

Acknowledgment

This work has been supported by the Operational Program Integrated Infrastructure, project Development of products by modification of natural substances and study of their multi-modal effects on COVID-19, ITMS2014+: 313011ATT2, co-financed by the European Regional Development Fund and project Development of models for enhancing the assessment of the efficacy of drugs and substances with potential in the treatment of COVID-19 (BIOVID-19), ITMS2014+: 313011AVG3, co-financed by the European Regional Development Fund.

ISBN 978-80-89991-14-3