30th Anniversary of the ISP

eBook of Abstracts

Centre of Experimental Medicine
Slovak Academy of Sciences, Bratislava, Slovakia

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PREFACE

Dear colleagues and friends,

***International Society for Pathophysiology* is celebrating its 30th anniversary!**

During its existence the ISP has been getting together researchers, clinicians, leaders and experts from research institutions and business companies from all around the world and provides a unique opportunity for global networking in the pathophysiology. The Society provides a comprehensive platform to discuss hot topics in pathophysiology, to identify new targets for diagnosis and treatment of human diseases as well as to define new research trends.

I am very pleased that this important anniversary coincided with my presidency. How else can we reflect on the thirty years of successful operation other than to review the latest results we have achieved in the last period. The two-day meeting dedicated to the 30 th anniversary of ISP consists of keynote review lectures by experts in pathophysiology as well as short presentations of experts and PhD students in various fields of pathophysiology. During the meeting we will be able to listen and discuss lectures from the field of cardiovascular system also from the point of view of pharmacological model of COVID-19, neurophysiology, metabolism, respiration, and redox signalling. The latest information about our journal *Pathophysiology* and next ISP Congress in Moscow will not be missing either. I believe that you find the program attractive and inspirative.

I'm looking forward to seeing you though only online.

Olga Pechanova
ISP President

METHYLENE BLUE REDUCES MAO-RELATED OXIDATIVE STRESS IN HUMAN EPICARDIAL TISSUE

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Coronary heart disease (CHD) is the leading cause of mortality due to myocardial infarction and of morbidity due to heart failure. Perivascular adipose tissue (PVAT) is essential in regulation and maintenance of vascular tone, remodeling, and endothelial function, while in obesity PVAT expresses a higher inflammatory profile, releasing angiogenic factors and inducing the proliferation of vascular smooth muscle cells leading to endothelial dysfunction and atherosclerosis. Monoamine oxidases (MAOs) are known as mitochondrial contributors to ROS production in the cardiovascular system and even though it was observed an increased MAO activity in obese animals, the translation of this observation in humans was not studied so far. Abnormal cardiac energy metabolism and mitochondrial dysfunction are key factors in cardiomyopathy, thus protecting mitochondrial function represents a research priority for the academic community worldwide. Because mitochondrial function is dependent on redox components, drugs with redox activity have been increasingly used as mitochondrial modulators to enhance energy production and decrease oxidative stress. One pharmacological agent, methylene blue (MB), known as an inhibitor of MAOs activity, has the ability to bypass mitochondrial defects, hence, there are no data about its effects on cardiovascular adipose tissue in coronary patients. In this view, the aim of the present study was to assess the effects of MB in preventing oxidative stress in coronary patients by modulating MAOs activity in PVAT obtained from patients subjected to cardiac surgery. Adipose tissue samples were isolated during heart surgical intervention, transferred to the laboratory, incubated with methylene blue (MB, 24h, 0.1 µM) and used for ROS measurements (techniques with ferrous oxidation xylenol and dihydroethidium), qRT-PCR and confocal microscopy (immune-fluorescence) studies. Our data revealed that human PVAT contains both MAO isoforms, with the predominance of MAO-A. Incubation with MB was able to reduce MAO expression together with generation of ROS; the effects on ROS generation were augmented by co-incubation with serotonin (10 µM; MAO-A substrate). In conclusion, MAOs might contribute via H_2O_2 generation to the dysfunctional process of the perivascular adipose tissue and MB is able to block the deleterious effects of MAO-related oxidative stress.

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Key words: Methylene blue; Perivascular adipose tissue; Monoamine oxidases; Oxidative stress.

THE EFFECT OF AN ANGIOTENSINE-CONVERTING ENZYME 2 INHIBITOR ON THE BLOOD PRESSURE AND VASOMOTOR RESPONSES IN EXPERIMENTAL HYPERTENSION

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It has been reported that some pre-existing cardiovascular pathologies belong to the risk factors predisposing the patients with SARS-CoV-2 infection to serious complications. It seems that the extracellular enzymatically active domain of full-length transmembrane anchored angiotensin-converting enzyme 2 (ACE2) is the receptor for the spike protein of SARS-CoV-2. Binding of the virus to this ACE2 results in dysfunction of Mas receptor-mediate pathways in the vasculature. This study was aimed to describe the effect of ACE2 inhibition on the vasoactive responses of the thoracic aorta (TA) and integrated pressure response of the cardiovascular system in essential hypertension.

Adult spontaneously hypertensive rats (SHR) were included into this study which were 14 days treated by ACE2 inhibitor, MLN-4760, due osmotic minipumps (1 mg/kg/day, s.c.). The vasoactive responses of TA were recorded by sensors of isometric tension (n=8 for each groups). The integrated pressure responses were followed in rats under general inhalation anesthesia (2,5 % of isofluran), the jugular vein was cannulated for drug application and the pressure transducer was placed into the carotic artery.

The MLN-4760 treatment did not alter the endothelium-derived vasorelaxation (EDVR) (acetylcholine, Ach, 10^{-10} – 10^{-5} mol/L) of TA rings. Similarly, the maximum force of the adrenergic contraction (noradrenaline, NA, 10^{-10} – 10^{-5} mol/L) was not changed after MLN-4760 treatment, however it shifted the concentration-response curve to exogenous noradrenaline to the left, the values of EC_{50} was also increased in MLN-4760-treated group. The acute inhibition of Mas receptors (A-799 trifluoroacetate salt, 10^{-5} mol/L), reduced the EDVR of TA, in both control SHRs and MLN-4760- treated SHRs. On the other hand, it significantly increased the contractile response to NA only in SHRs treated with MLN-4760. The NO synthase inhibition by L-NAME (10^{-5} mol/L) significantly inhibited the EDVR of TA similarly in both groups. However, it increased the NA-induced contraction only in SHRs treated with MLN-4760. The incubation with a H2S scavenger (bismuth(III) subsalicylate, BSC, 10^{-5} mol/L)significantly decreased EDVR only in SHRs treated with MLN-4760. The scavenger application increased the contractile response in both experimental groups. The MLN-4760 treatment did not alter the baseline values of mean arterial pressure in vivo, similarly had no effect on the integrated pressure response either to Ach (1μg/kg) or NA (1μg/kg). The acute pre-treatment with L-NAME (30 mg/kg; NO-synthase inhibitor) significantly increased blood pressure as well as the hypotensive response induced by Ach similarly in both, control and MLN-4760 treated group. BSC (0.25 μg/kg) induced a mild hypotensive response in control group; however, it increased the mean blood pressure in MLN-4760-treated group.

Our results demonstrated that the low-dose administration of MNL-4760 did not evoke a significant alteration in the functional properties of the arterial wall in conduit as well as in resistant arteries. While the Mas receptor-related pathway was involved in maintaining both EDVR and contractility of isolated vessels in both groups, the proportion of NO and H_2S signalizations after MLN-4760 treatment changed to avoid from additional cardiovascular complications. Supported by PP-COVID-20-0043.

Fe_3O_4@PEG NANOPARTICLES ALTER IRON METABOLISM AND NITRIC OXIDE PRODUCTION IN THE LIVER OF SPONTANEOUSLY HYPERTENSIVE RATS

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We investigated the effects of polyethylene glycol (PEG)-coated magnetite nanoparticles (Fe_3O_4@PEG) with ~30 nm core size, ~51 nm hydrodynamic size on iron metabolism and nitric oxide (NO) production in the liver of Wistar–Kyoto (WKY) and spontaneously hypertensive rats (SHR). Fe_3O_4@PEG were administered intravenously, for two consecutive days at the dose of 2 mg Fe/kg/day dispersed in saline. Control rats were treated with saline only. Fe_3O_4@PEG significantly reduced the relative blood pressure (BP, calculated as the percentage change to basal level of the given group) of Fe_3O_4@PEG-treated SHR (SHRu) compared to both Fe_3O_4@PEG-treated WKY (WKYu) and saline-treated control SHR (SHRc). The Fe_3O_4@PEG content, determined by SQUID magnetometry and electron microscopy, was significantly elevated in the liver of SHRu vs. WKYu. Nitric oxide synthase (NOS) activity and eNOS and iNOS gene expressions were significantly increased in SHRu vs. SHRc and there was a significant positive correlation between NOS activity and eNOS and iNOS gene expressions, respectively, in the liver. Fe_3O_4@PEG also elevated DMT1 and FTH1 gene expression (considered main effects). Results showed that elevated incorporation of Fe_3O_4@PEG alters iron metabolism in the liver and lead to increase of NO production, due to activation of iNOS and eNOS gene expression. This can result in lower peripheral vascular resistance associated with BP decrease only in SHR. In conclusion, our findings suggest caution when using Fe_3O_4@PEG nanoparticles in hypertensive subjects, as they may alter iron metabolism and hepatic NO production followed by BP decrease.
Study was supported by the grants Nos. APVV-16-0263 and VEGA-2/0157/21.

THE EFFECT OF CEREBELLAR TRANSCRANIAL DIRECT CURRENT STIMULATION ON SEMANTIC MEMORY

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Abstract: Transcranial direct current stimulation (tDCS) is a safe non-invasive neurostimulatory method widely used in research and clinical practice. Modulation of neural activity via tDCS can be achieved by delivering a weak electric current between two surface electrodes, anode and cathode. While most of the tDCS protocols involve neocortical stimulation, recently its ability to modulate the cerebellum has been demonstrated as well. The cerebellum is a structure mainly associated with the coordination of motor functions. Currently, new insights about its involvement in various affective and cognitive processes have emerged. Within the cognitive domain, it has been linked to functions such as working memory, attention or verbal fluency. However, the exact contribution of the cerebellum to these processes remains unknown. Our study, therefore, aimed to investigate the role of the cerebellum in semantic memory with anodal and cathodal transcranial direct current stimulation (tDCS). The final cohort consisted of 140 healthy participants, randomly divided into three groups (anodal tDCS, cathodal tDCS and sham tDCS). Two semantic memory tasks were administered before and immediately after tDCS. The first task was the associative chain test where the participants were instructed to generate word associations and dissociations according to the specific rules. The second behavioural task was the semantic prediction task, involving finishing the sentences with two levels of predictability (highly predictable vs. unpredictable sentences). The duration of the stimulation was 20 minutes, with the electric current intensity of 2 mA. tDCS positively affected the generation of word associations without affecting any other parameter of lexical-semantic processes. Our results imply that tDCS can be successfully utilised to modulate the cerebellar function and that the cerebellum may be involved in some aspects of lexical-semantic processing and semantic memory. Implied results can be relevant in the context of growing evidence about the cerebellar role in several neuropsychiatric disorders accompanied by cognitive dysfunctions.

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EVALUATION OF MITOCHONDRIAL DYSFUNCTION AND OXIDATIVE STRESS IN THE SETTING OF PREECLAMPSIA

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Preeclampsia (PE) is the most severe complication of pregnancy with substantial burden of morbidity and mortality for both mother and neonate. While mild PE is associated with proteinuria, the severe forms of the disease also associates one or more of the following: thrombocytopenia, hemolysis, disseminated intravascular coagulation, impaired liver function, acute kidney injury, neurological complications and fetal growth restriction (FGR). Timing of PE recognizes 2 forms of the disease: early-onset PE, developed before 34 weeks of gestation (associated with fetal growth restriction) and late-onset PE after 34 weeks of gestation (normal or large for gestational age fetuses). A growing body of research has unequivocally demonstrated the role of mitochondrial dysfunction and oxidative stress as central mechanisms underlying the abnormal placentation in PE; however, the complex pathophysiology of PE is far from being fully elucidated and many data in the literature are controversial.

The present study was double-aimed: i) to assess mitochondrial respiratory function in platelets and isolated placental mitochondria and ii) to assess the oxidative stress in plasma and placental tissue. For the study of mitochondrial function, participants (n=57) were included in two groups: I) the platelet study group (n=33, with 3 subgroups: PE, healthy pregnancies, age-matched controls) and II) the placenta study group (n=24, with 3 subgroups: PE with or without FGR and healthy pregnancies). Mitochondrial respiration of isolated platelets and placental mitochondria) was assessed by means of high-resolution respirometry according to a protocol adapted to measure complex I (glutamate + malate) and complex II-dependent (succinate) respiration. The main respiratory parameters were: routine respiration, active respiration (after ADP addition) and maximal uncoupled respiration (in the presence of FCCP). Systemic oxidative stress was evaluated in 39 subjects: 20 participants (PE and healthy pregnancy) while 19 participants (healthy, mild or severe PE) were evaluated for the local oxidative stress in placental tissue. Systemic oxidative stress was assessed in plasma using the Diacron equipment, measuring both reactive oxygen metabolites (d-ROMs) and plasma biological antioxidant capacity (BAP). Placental samples (central and peripheral) were collected and stored at -80; cryosections were incubated with dihydroethidium and analysed in confocal microscopy.

Platelets harvested from preeclamptic pregnancies showed a lower coupled and uncoupled respiration as compared to both healthy pregnancies and controls. Placental mitochondria isolated from PE associated with FGR showed a decrease in both active and maximal uncoupled respiration for both mitochondrial complexes. At variance, placental mitochondria isolated from PE without FGR showed an increase in both active and maximal uncoupled respiration. Systemic oxidative

stress was decreased in preeclamptic pregnancies but was associated with a significant increase in plasma antioxidant capacity. The severe forms of preeclampsia presented increased values of the placental oxidative stress in both placental regions as compared to mild preeclampsia.
In conclusion, preeclampsia is characterized by mitochondrial dysfunction in both platelets and placentas, which additionally associates in severe forms an increase in local oxidative stress.

PATHOPHYSIOLOGY – PRESENT AND FUTURE IN THE INTERNATIONAL CONTEXT

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Pathophysiology represents an integrative discipline which studies the alteration of the physiological functions of the body focusing on the causes, mechanisms underlying the development of the disease, the functional consequences and clinical signs. Also contributes to understanding medical terminology and it is essential in establishing therapeutic strategy. Pathophysiology has an interdisciplinary character, linked with another fundamental disciplines (biochemistry, physiology, pathology) and represents also a bridge between preclinical and clinical disciplines.
In our university, in the Pathophysiology Department the teaching activity during both lectures and laboratory works is student-centred, permanently focusing on an interactive relationship with our students, to motivate them to correlate the underlying mechanisms of the diseases (presented during the lecture) with the laboratory (paraclinical alterations detailed and exemplified by case studies presented at the practical works). It is important that students acquire all perspectives about the concept of disease and develop an analytical medical thinking, essential for an accurate diagnosis and for a rigorous scientific treatment in everyday practice.
In the last years, the scientific research has mainly focused on attracting funds necessary for the study of the endothelial and mitochondrial (dys)function as potential therapeutic targets in various pathologies: cardio-metabolic, renal, old-age and carcinogenesis. At the same time, there have been other important research partnerships with prestigious teams in the country and abroad focusing on the study of oxidative stress, the contribution of arterial rigidity to the pathogenesis of cardiovascular diseases with or without comorbidity factors and, more recently, on the study of purinergic signaling.

PAIN: ON THE BORDERLINE BETWEEN PHYSIOLOGY AND PATHOPHYSIOLOGY

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Acute or chronic pain has a different pathophysiological mechanisms according with their different etiologies. The borderline between physiological and pathophysiological outcome is up to the various neuroplasticity mechanisms associated with pain. In chronic pain neuroplasticity occurs in the context of structural remodelling and reorganisation of synapses, potentially contributing to the long-term nature of chronic pain. Chronic pain is not only simply following acute pain, multiple and complex mechanisms can contribute to a chronic pain onset and evolution. Inflammation is one of the most important contributor to chronic pain mechanism. Modulatory mechanisms of inflammation can have a benefic effect on pain perception and pain associate behaviour. This review discuss pain modulatory mechanisms in experimental studies (on animal models) and the contribution of experimental studies to understanding modulatory mechanism in clinical practice. The borderline between physiological and pathological condition of pain reception, transmision and perception are also discussed.

THE EFFECTS OF LOW DOSE ACE2 INHIBITOR MLN-4760 ON BLOOD PRESSURE, ADIPOSITY AND RENIN-ANGIOTENSIN SYSTEM IN SPONTANEOUSLY HYPERTENSIVE RATS

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Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), was declared as a global pandemic. Although the clinical manifestations of COVID-19 are dominated by respiratory symptoms, some patients have severe cardiovascular damage. In addition, some pre-existing cardiovascular pathologies, such as hypertension, are the risk factors which predispose the patients with SARS-CoV-2 infection to serious complications or even to death. Viral infections are dependent on cellular entry via the host protein angiotensin-converting enzyme-2 (ACE2), which represents the receptor for spike protein of SARS-CoV-2. This alters downstream signalling of angiotensin 1-7 (Ang 1-7), the main product of ACE2 action. In uninfected cells, Ang 1-7, binds to the Mas receptors within the cardiovascular system and

reveals essential protective effects. Since binding of the virus to transmembrane ACE2 could result in dysfunction of Mas receptor-mediate pathways in the vasculature it is crucial to reveal the role of ACE2 inhibition especially within pre-existing cardiovascular pathologies. In our project we imitated the inhibition of ACE2-mediated signalling using ACE2 inhibitor MLN-4760 with the aim to investigate to what degree is the inhibition of ACE2 detrimental for cardiovascular system of hypertensive subjects. As an animal model we used spontaneously hypertensive rats (SHR), which represent a model of human essential hypertension. SHR are characterized by increased systolic blood pressure, cardiac and vascular wall hypertrophy, elevated systemic resistance, endothelial dysfunction, altered arterial contractility, and have underlying lung abnormalities such as haemorrhage, inflammation, and oxidative burden. The aim of this study was to evaluate the effect of MLN-4760 on blood pressure, adiposity, and its relationship to plasmatic angiotensins. We also investigated the role of renin-angiotensin system (RAS) in the maintenance of blood pressure in vivo. Low dose of MLN-4760 (1 mg/kg/day) was diluted in 10% DMSO at the dose of 1 mg/kg/day and infused subcutaneously for 14 days by Alzet osmotic minipumps. The equilibrium levels of individual angiotensins in plasma were quantified by liquid chromatography mass spectrometry/mass-spectroscopy. Integrated pressure responses of the cardiovascular system induced by captopril were monitored using a probe placed into the right carotid artery. The systolic blood pressure, plasma level of individual angiotensins, plasma renin and ACE activities were unchanged in SHR treated with MLN-4760. On the other hand, the ACE2 inhibition resulted in a significant increase of body weight increment, elevated adiposity, and strong tendency of increased glucose plasma level and reduced alternative RAS activity. Moreover, the body weight and visceral fat mass negatively correlated with plasmatic alternative RAS activity and Ang1-7 suggesting the importance of alternative arm of RAS in regulation of adiposity. We also confirmed that ACE inhibitor captopril (10 mg/kg) induced a hypotensive response in both groups, however the decrease in mean blood pressure was significantly lower in MLN-treated rats suggesting that contribution of Ang II to blood pressure maintenance could be reduced or that protective bradykinin- mediated effects associated with captopril action were impaired. Our results suggest that favourable effect of Ang1-7 on adiposity is diminished when ACE2 is inhibited. Moreover, the inhibition with low dose of ACE2 could lead to disharmony of RAS in the blood pressure maintenance.

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HMGB1: A POTENTIAL TARGET IN EXPERIMENTAL MYOCARDIAL INFARCTION

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Introduction: Myocardial infarction (MI) remains a leading cause of morbidity and mortality among all cardiovascular diseases over the world. High mobility group box 1 (HMGB1) is a non-histone chromosomal protein with multiple cardioprotective effects. Besides its nuclear role, HMGB1 participates in interaction including the production of proinflammatory cytokines. The aim of the study was to evaluate the effects of anti-HMGB1 protein on biochemical and morphological parameters after experimental MI.
Methods: 12-week-old male WKY rats used for the study were divided into following groups: sham operated WKY without MI, WKY with MI, WKY + IM+ anti-HMGB1 protein. In vivo model of experimental MI was induced by ligation of the left descending coronary artery and lasted 20 min. Prior to reperfusion anti-HMGB1 protein was administrated i.v. 7 days after MI, nitric oxide synthase (NOS) activity was determined by conversion of ^{3}[H] Arginine to ^{3}[H] Citrulline in the aorta and ischemic, border and non-ischemic region of the heart. NFκB, iNOS and eNOS expression was determined by Western blot. For morphological parameters, the hearts were excised and used for TTC-staining procedure. Cytokine levels were investigated using the Bio-Plex Pro Cytokine kit in the plasma. Concentration of conjugated dienes was measured spectrophotometrically in the heart.
Results: Anti-HMGB1 protein increased NOS activity in both ischemic and border heart zone, as well as in the aorta, on the other hand NOS activity was not changed in non-ischemic part of the heart. The same pattern was found in eNOS expression level. The protein administration significantly decreased NFκB expression in MI part of the heart, as well as TNF-alpha and IL-6 level in plasma. Simultaneously, anti HMGB1 protein decreased MI part as well as border region of the heart.
Discussion: Considering the results by using a rat model of experimentally induced MI, HMGB1 protein is a promising molecule for reduction the negative effects of the myocardium infarction, as well as a promising agent for the treatment of cardiovascular diseases.
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ANTIOXIDANT EFFECTS OF LONICERA CAERULEA L. ON CARDIOMETABOLIC PARAMETERS AND NO/ROS BALANCE IN OBESE ZUCKER RATS

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One of the promising sources of polyphenols, Lonicera caerulea L. (Loni) represents therapeutical potential in cardiovascular diseases. We aimed to study the effects of Loni and coenzyme Q10 (CoQ10) on selected cardiometabolic parameters and NO/ROS balance in obese Zucker rats.
Male Zucker rats were divided into the control group and groups treated with CoQ10 (30 mg/kg/day) or Loni (5 g/kg/day) for 6 weeks. Blood pressure, body weight, heart weight, and plasma lipid profile were determined. NOS activity and protein expressions of eNOS, SOD, NADPH oxidase, and NF-kappa B were measured in the heart and aorta.
Neither body weight nor blood pressure were significantly changed after six weeks of Loni or CoQ10 treatment. Both Loni and CoQ10 decreased the plasma LDL level. Moreover, Loni decreased the total cholesterol level. The total NOS activity did not change in the heart after the treatments. However, in the aorta, Loni treatment increased NOS activity and protein expression of SOD and decreased expressions of NADPH oxidase and NF-kappa B compared to both the control and CoQ10 groups. There were no changes in the eNOS protein expression within the groups.
The antioxidant effect of Lonicera caerulea L. was demonstrated in several animal models of cardiometabolic diseases. We first demonstrated this effect in obese Zucker rats. Since Lonicera caerulea L. is rich in antioxidant anthocyanins and low in sugar, which was demonstrated also in our study, this berry can be suggested as a supplement treatment in dyslipidemia and other cardiometabolic disorders.

THE EFFECT OF INHIBITION OF HYDROGEN SULFIDE PRODUCTION ON THE REACTIVITY OF THE CARDIOVASCULAR SYSTEM.

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Hydrogen sulfide (H_2S) plays an important role in modulating of the vasoactive responses of the cardiovascular system. The lack of information on the mutual interaction of endogenously produced NO and H_2S led us to investigate the effect of chronic H_2S inhibition in combination with acute NO deficiency on blood pressure responses of peripheral arterial stream. At the same time, we have investigated the role of endogenously produced H_2S in the vasoactive responses of different types of arteries in an in vitro study.

The 6-week inhibition of H_2S production was induced by propargylglycine (PPG, 30 mg/kg/day dissolved in physiological solution, intraperitoneally, i.p. n=10) at the beginning of the 10th week of Wistar rats. Systolic blood pressure (sBP) was measured weekly by the plethysmographic method on the tail artery. Integrated responses of the cardiovascular system were monitored in anaesthetized rats (mixture of Zoletil 100; 4.0 mg/100 g bw; and xylazin; 0.5 mg/100 g bw) via a fiber-optic probe placed into the right carotid artery and connected to a pressure transducer. Vasoactive substances: noradrenaline (NA; 0,1µg/kg), acetylcholine (ACH; 0,1µg/kg) and sodium sulphide (Na2S; 8µM/kg) were administered via the right jugular vein and blood pressure responses were studied before and after acute inhibition of endothelial NO-synthase (eNOS) with L-N^G-nitro arginine methyl ester (L-NAME). At the same time, the participation of endogenous H_2S in the basic vasoactive responses of the thoracic aorta (TA) and mesenteric artery (MA) was followed in acute experiments as changes in isometric tension.

Six weeks of PPG treatment did not elicit changes in sBP and did not alter the vasoactive response to NA and Na_2S, although the vasodepressor response to ACH was reduced compared with the control group. Acute inhibition with L-NAME elicited a reduced vasopressor response in the PPG group. Moreover, acute application of L-NAME evoked an enhanced vasodepressor response to ACH and Na_2S in both groups, although this enhancement was lower for ACH and higher for Na_2S in the PPG group. The results of acute H_2S deficiency on the vasoactive responses of isolated arteries to NA showed an increase in basal tone in MA and increased sensitivity of adrenergic receptors in TA, whereas endothelium-dependent vasorelaxation was unaffected.

Long-term administration of PPG demonstrated the modulatory role of endogenous H_2S on endothelial NO bioavailability in peripheral arteries. Our results showed that endogenously produced H_2S has different effects depending on the type of artery. Whereas the acute H_2S deficiency led to an increase in active tone of elastic arteries (TA) it increased the basal tone of muscular arteries (MA). The results of our study confirmed that unlike in large and medium-sized conduit arteries, mediators other than endothelial NO are present in resistant vascular beds. In combined H_2S and NO deficiency, activation of H_2S-independent compensatory mechanisms plays an important role in maintaining vasodilator responses of the cardiovascular system.

LOSS OF NPPA-AS1 PROMOTES HEART REGENERATION BY STABILIZING SFPQ-NONO HETEROMER-INDUCED DNA REPAIR

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Rationale: The role of long non-coding RNA (lncRNA) in endogenous cardiac regeneration remains largely elusive. The neonatal mammalian heart is capable of full regeneration following injury through proliferation of preexisting cardiomyocytes. This fact allows the exploration of the roles of critical lncRNAs in the regulation of post-injury cardiac regeneration.

Objective: We aimed to characterize the lncRNA transcriptome in neonatal mice with apical resection (AR) and define the roles of novel lncRNAs in cardiomyocyte proliferation after postnatal and adult injury.

Methods and Results: RNA sequencing was performed in a cardiac regeneration model by apical resection (AR) of the left ventricle of neonatal mice. LncRNA-deleted mice, generated by the CRISPR genome-editing system, were used to determine the role of lncRNAs in cardiac repair after myocardial infarction (MI). We identified a novel lncRNA, named NPPA-AS1, which significantly reduced after AR and negatively affected cardiomyocyte proliferation. NPPA-AS1 deletion did not affect heart development but was sufficient to prolong the postnatal window of regeneration after AR. In adult mice, NPPA-AS1 deletion improved cardiac function and reduced infarct size in post-MI hearts, which were associated with significant improvement in cardiomyocyte proliferation. Further analysis showed that NPPA-AS1 interacted with DNA repair-related molecule SFPQ (Splicing factor, proline- and glutamine-rich). SFPQ-NONO (Non-POU domain-containing octamer-binding protein) heteromer is required for double-strand DNA break repair, but NPPA-AS1 competed with their binding to DNA due to overlapping binding sites. Indeed, NPPA-AS1 deletion promoted the binding of SFPQ-NONO heteromer, decreased DNA damage, and activated cardiomyocyte cell-cycle re-entry.

Conclusions: Loss of NPPA-AS1 promoted cardiomyocyte proliferation by stabilizing SFPQ-NONO heteromer-induced DNA repair and exerted a therapeutic effect against MI in adult mice. Thus, NPPA-AS1 may be a novel target for stimulating cardiac regeneration to treat MI.

THE EFFECT OF PERIVASCULAR ADIPOSE TISSUE AND ENDOGENOUS HYDROGEN SULFIDE IN VASOACTIVE RESPONSES OF ISOLATED THORACIC ARTERIES IN NORMOTENSIVE AND SPONTANEOUSLY HYPERTENSIVE RATS

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Perivascular adipose tissue (PVAT) plays an important role in the regulation of cardiovascular system (1). One of the crucial substances produced by both vascular wall and PVAT is hydrogen sulfide (H_2S) which reveals a biphasic vasomotor effect on cardiovascular system. Several authors have confirmed the relationship between PVAT and H_2S in association with the etiopathogenesis of different cardiovascular and metabolic diseases (1). The aim of this study was to evaluate the mutual relationship between PVAT and endogenous H_2S in the contractile and relaxant responses of thoracic aorta (TA) isolated from adult normotensive (Wistar) rats and spontaneous hypertensive rats (SHRs). Systolic blood pressure (sBP) was measured by plethysmographic method, and the contractile and relaxant abilities of TA were evaluated after application of exogenous noradrenaline (Na) and acetylcholine (Ach) in TA with preserved or denuded PVAT. To inhibit the endogenous H_2S production, the inhibitor of cystathionine γ-lyase, propargylglycine, was used.

The blood pressure was significantly higher in SHR compared to Wistar rats, which was associated with cardiac hypertrophy and increased contractility of TA. Although there were no differences in the amount of retroperitoneal fat between strains, the increased plasmatic level of triacylglycerol has been declared in SHR.

Regardless of the strain, PVAT revealed anti-contractile effect on the vasoconstrictor responses induced by exogenous NA and the lowest contractile response was observed in Wistar rats with preserved PVAT. In both, Wistar and SHRs, PVAT worsened the endothelial-dependent vasorelaxant response induced by Ach. In Wistar rats, H_2S produced by the vascular wall as well as PVAT did not participate on the vasoconstrictor response induced by exogenous NA. However, in SHR, H_2S produced by both, the vascular wall and PVAT had a pro-contractile effect on the vasoconstrictor response induced by exogenous NA. In Wistar rats, H_2S produced by the vascular wall as well as PVAT was not involved in the endothelial-dependent vasorelaxant responses. On the other hand, even if H_2S produced by PVAT did not contribute to endothelium-dependent vasorelaxant responses, H_2S produced by the vascular wall had a pro-relaxant effect in SHR.

Our results confirmed that although PVAT of TA under normotensive and hypertensive conditions aggravated endothelial function, it revealed anti-contractile effect mediated by a release of unknown factor. Endogenously produced H_2S manifested a dual effect depending on the type of signaling pathway triggered. Unlike in Wistar rats, in SHR H_2S produced by PVAT as well as vascular wall had pro-contractile effect suggesting its contribution to pathological changes of essential hypertension. On the other hand, we confirmed that H_2S produced by TA vascular wall of hypertensive rats had pro-relaxation effect and could represent a form of compensatory mechanisms to balance impaired vascular tone regulation.

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MEMBRANE-DELIMITED SIGNALING AND CYTOSOLIC ACTION OF MG53 PRESERVE HEPATOCYTE INTEGRITY DURING DRUG-INDUCED LIVER INJURY

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Background & Aims: Drug-induced liver injury (DILI) is a leading cause of acute liver failure, and treatment of DILI remains a challenge. Muscle-derived protective factor MG53 plays an essential role in injury repair, but whether MG53, released from skeletal muscle into circulation, can function as a myokine and promote repair of injury in liver tissues remains undetermined.
Methods: The functions of MG53 during DILI was investigated by giving recombinant MG53 protein (rhMG53) exogenously and using mice with deletion of MG53 or RIPK3. Live cell imaging, histological, biochemical and molecular studies were used to investigate the mechanisms that underlie the extracellular and intracellular action of rhMG53 in liver protection.
Results: While healthy human and animal livers do not contain endogenous MG53 protein, patients with obstructive cholestatic and liver transplant diseases show increased accumulation of MG53 in the liver. Mice with ablated MG53 show higher susceptibility to DILI, while rhMG53 protein can treat several DILI in mice, including acetaminophen, tetracycline, concanavalin A, carbontetrachloride, and thioacetamide. Circulating MG53 protects hepatocytes from injury through direct interaction with MLKL at the plasma membrane. Moreover, extracellular MG53 can enter hepatocytes and act as an E3-ligase to mitigate RIPK3-mediated MLKL phosphorylation and membrane translocation.

Conclusions: Our data show that the membrane-delimited signaling and cytosolic dual action of MG53 effectively preserve hepatocyte integrity during DILI, and rhMG53 may be a potential treatment option for patients with DILI.

Keywords: APAP, drug-induced liver injury, rhMG53, RIPK3, MLKL.

RELAXANT EFFECT OF PULMONARY SURFACTANT ON THE AIRWAY SMOOTH MUSCLE OF ALBUMIN-SENSITIZED GUINEA PIGS

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Introduction: In addition to well defined functions of pulmonary surfactant in the alveoli as reduction of surface tension, prevention of alveolar collapse and lung edema and immune function, relaxing effect on the airway smooth muscle (ASM) in vitro has been described (Koetzler et al., 2006). In our previous study, exogenous pulmonary surfactant relaxed precontracted bronchial strips containing smooth muscle in vitro (Topercerova et al., 2019). It is not known if such effect is also exerted by surfactant on the ASM under pathological conditions of hyperresponsiveness. Aim: To test the hypothesis that pulmonary surfactant may relax the airway smooth muscle in ovalbumin-sensitization. Methods: The experiments were performed on 18 adult guinea pigs (Dunkin Hartley, males, body weight approx. 300 g) using in vitro method of organ baths (Mokry et al. 2006) Tracheal and lung tissue strips of healthy animals and animals sensitized by ovalbumin (OVA) (albumin chicken egg) for fourteen days were placed into organ chambers with Krebs-Henseleit solution. Continual temperature was maintained at 37.0 °C ± 0.5 °C and aeration by pneumoxide (95% O_2 and 5% CO_2) to provide physiological pH of 7.5 ± 0.1. Tension of ASM was evaluated at cumulative doses of methacholine or histamine. Relaxant effect of exogenous pulmonary surfactant (Curosurf®, Chiesi Farmaceutici, Parma, Italy) was evaluated in presence of indomethacin. Results: Muscle tension of tracheal tissue strip ($p<0,05$) and lung tissue strip ($p<0,01$) of OVA-sensitized animals precontracted with methacholine was significantly reduced by surfactant. Moreover, surfactant prevented the hyperresponsive ASM from contracting when methacholine was applied ($p<0,05$). Indomethacin had a tendency to block the relaxant effect of surfactant.
Conclusion: In vitro, pulmonary surfactant relaxes airway smooth muscle of ovalbumin-sensitized guinea pigs. This finding emphasizes the role of surfactant in the physiology of the airways and supports the therapeutic potential of exogenous surfactant in asthma and chronic obstructive pulmonary disease. Acknowledgements: The study was supported by VEGA 1/0055/19 and APVV-17-0250.
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BIOLOGICAL IMPERFECTION OF HOMO AS A CENTRAL CONCEPT OF PATHOPHYSIOLOGY AND FUNDAMENT OF HUMANISM: PROBLEM OF CONTROVERSIES BETWEEN CLINICAL AND SCIENTIFIC REASONING

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The compensatory/adaptive mechanisms laid down by nature and programmed in the genetic apparatus of the body are fundamentally imperfect, they have only a relative protective effectiveness, adaptation based on them always has some costs in the form of undesirable consequences of their action for certain organs. In Normal Physiology the central subject is adaptation and health understood as fitness with a situation, but in Pathophysiology the core element is a price of adaptation and disease understood as a measure of discrepancy with the situation. However, the ability to get sick, depending on this feature of defense mechanisms, is inherent in our nature and it is this particular facet of the body which makes us human. An absolutely perfect creature, making no mistakes, neither human nor humanistic. The imperfection and secondary pathogenicity of natural defence (autopathokinesis) is a central idea of Pathophysiology, a science constructed around this statement regardless of physiological, biochemical, immunological, biophysical or clinical methods which Pathophysiology may use. The programmed defense reactions are themselves pathogenic and give rise to secondary disturbances, often incommensurately more harmful than the action of the primary damaging factor itself. Any disease is a mosaic of pathological processes which should not be always interpreted as a harmonic defensive orchestra. On the contrary, automatic defensive stereotypes of different levels (local, driven by juxta-, para- and autocrine signals within the foci of pathological processes versus systemic, controlled by central neuroendocrine mechanisms) being parallel in course of disease may come into conflict contradicting each other and increasing the costs of adaptation, making it dangerous. The conflict of local and systemic mechanisms devaluating their effectiveness is common both in acute (hemodynamic shock) and in chronic (metabolic syndrome and autoimmunity) forms of pathology. This raises the question of health as the biological equivalent of freedom and gives birth to a number of philosophical and ethical contradictions inherent in scientific and medical activities. The fundamental unattainability or unacceptably high cost of the main goals that the public conscience sets for medicine lies at the heart of the discrepancy between scientific reasoning and clinical thinking. Pathophysiology is a key element of medical education bridging these two systems of reasoning and creating their symbiosis in a learning mind. The goals, conditions and canons of these two interrelated, but different types of intellectual activity are compared in relation to the public perception of scientific and medical activity and the problems of experimentophobia and neo-obscurantism which became actual nowadays. Medicine as a part of human culture related to health and disease is not only a science and organically includes unscientific elements. The thesis about the degeneration of evidence-based medicine and its transformation into an anti-scientific one is substantiated. Ethical systems that blur the line between norm and pathology and ignore fundamental biological differences between people are criticized. The role of Pathophysiology as a prerequisite and king-stone of translational medicine is emphasized.

Key words: scientific thinking, clinical reasoning, typical pathological processes, primary alteration, secondary alteration, health, disease, adaptation, autopathokinesis.

MAY THE CELLULAR MEMORY BE STORED BY MEANS OF THE EXTRACELLULAR VESICLES?

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It is known that the Extracellular Vesicles (EVs) contain proteins, strips of nucleic acids and lipids. They are nowadays established as important lines of communication between cells. The EVs carry information as fragments of DNA, microRNA, messengerRNA and other non-coding RNAs. Exosomes, as one type of EVs, are known to facilitate intercellular communication processes between cells in proximity as well as for distant cells too. They were found in each internal body fluid as well. The EVs play an essential role not only in normal physiology, but also in pathological intercellular communication. As it is stressed in contemporal scientific articles EV research has become common-place in every field of biomedicine, being explored as diagnostics and therapeutics. Already in the 60-ties of the last century Engel (1962) formulated the bio-psycho-social model that the health and illness are consequences of the interplay of biological, psychological, and social factors. In spite of this, contemporary medicine and medical research concentrates mostly upon the diagnostics and therapy of the biological basis of the illnesses. The new data on the EVs functions may also help for research of psychosocial consequences determining the man´s health. As an example here we will point to the difference between the biological basis of something we can call "inner" and "outer" memory. In the first case we mean the memories stemming from our organism, maybe even from our organs only. That is the memories evoked from physiological mechanisms acting inside of our body. These memories are stored without the intentional effort. The information may be stored in exosomes. In the second case we took into account the memories evoked by the visual, acoustical, haptic, olfactory experiences etc. That is the memories based on the previous information coming from the external environment, the information focused by our attention at the given moment. The question is if our „inner" memory may influence our psychosocially determined mental set? Are the exosomes engaged in its "revival"? Why not and if yes then how?

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THE PROTECTION OF AKT/HIP-55/MAP4K1 SIGNALING PATHWAY IN MYOCARDIAL INFARCTION INJURY

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Ischemic heart disease is a leading cause of death worldwide. Myocardial infarction (MI) results in cardiac damage due to cell death and insufficient cardiomyocyte self-renewal. Therefore, early endogenous protection against myocardial death is crucial to limit infarct size and improve clinical outcomes. However, knowledge of fundamental mechanisms of cardiomyocyte survival and regulated cell death in MI remains incomplete.

In the present study, we report an adaptor protein HIP-55 is identified as a new AKT substrate which mediate AKT-driven negative regulatory system that attenuates MAP4K1 cell death pathway against cardiomyocyte death after MI. The expression of HIP-55 is induced in MI. Genetic deletion of HIP-55 increases MI injury, whereas cardiac-specific overexpression of HIP-55 significantly alleviates the injury. Mechanistically, HIP-55 interacts with AKT and MAP4K1 to form a HIP-55 signalosome, where AKT phosphorylates HIP-55 at S269/T291 sites and further HIP-55 directs AKT signaling to negatively regulate the MAP4K1 pathway against MI injury in a site-specific manner. S269A/T291A-mutated HIP-55 (HIP-55AA), which is defective in AKT phosphorylation and decreases significantly the interaction between HIP-55 and MAP4K1, fails to inhibit the MAP4K1 cell death pathway. In line with this mechanism, cardiac-specific overexpression of HIP-55AA mouse also fails to protect cardiomyocytes against MI-induced injury in vivo. These findings define HIP-55 acts as a hub protein for the integration of the AKT/HIP-55/MAP4K1 signalosome function against MI injury. HIP-55 may be a new therapeutic target in the setting of acute myocardial damage.

THE GINSENG VALUE IN STRESS AND ITS USE IN THE CURRENT CONTEXT

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Panax ginseng (GSG) is a plant well known and used in the Far East, used for over 2000 years, especially in China, Japan and Korea. It is known that GSG is a very important adaptogen, that can increase long-term resistance to stress and disease and thus improve life expectancy.
GSG has been used as an anti-stress agent, due to ginsenosides, its main active components. Also, GSG saponins have been shown to play an important role in the anxiolytic effects of GSG, so GSG may be useful in the treatment of anxiety.
Of the GSG varieties, the Korean red GSG is the best known and considered very valuable for health and stress modulation. Thus, Korean red GSG could help stabilize the sympathetic nervous system and improve cognition in people with high stress.
The present paper aims at the importance of GSG in stress modulation, both psychological and the oxidative one, as well as highlighting the benefits of using GSG in the current period we are going through.
Key words: Panex Ginseng, adaptog

DIFFERENT EFFECT OF MLN-4760 ON MRNA EXPRESSION OF GENES ASSOCIATED WITH OXIDATIVE STRESS, INFLAMMATION AND NITRIC OXIDE PRODUCTION IN THE HEART AND LIVER OF SPONTANEOUSLY HYPERTENSIVE RATS

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) enters the cells via binding to angiotensin converting enzyme 2 (ACE2) on cellular membrane. Binding of SARS-CoV-2 to the ACE2 protein leads to disruption of the renin-angiotensin system, which may lead to COVID-19 progression, especially in patients with comorbidities, namely hypertension. Our study investigated the effects of MLN-4760 (MLN), a selective ACE2 inhibitor, on superoxide and nitric oxide (NO) productions in the left heart ventricle (LHV) and liver of spontaneously hypertensive rats (SHR). We also investigated the genomic effects of MLN administration on the expression of genes associated with NO and superoxide production as well as inflammation. MLN was administered subcutaneously using Alzet osmotic minipump (1 mg/kg body weight/day, dissolved in 10% DMSO solution) for 14 days. Control rats were treated with 10% DMSO solution only. Superoxide production was analysed by determination of lucigenin-enhanced chemiluminescence. NO synthase (NOS) activity was determined by conversion of 3H-arginine to 3H-citruline. Gene expressions were determined by qPCR.

We found significantly ($p < 0.05$) increased NO production and decreased superoxide production in the liver of MLN-treated rats compared to the control group. However, the increased NO production and reduce superoxide production after MLN treatment were not associated with changes in expression of genes involved NO production (eNOS, iNOS and nNOS), genes involved in antioxidant defence system (SOD1, GPX4 and HO-1) or genes associated with inflammation (IL-1β, TNF-α, hepcidin). In contrast, MLN administration did not affect superoxide and NO formation in LHV. Interestingly, we detected significantly ($p < 0.05$) increased expression of genes associated with NO production (iNOS and nNOS), inflammation (IL-1β) and antioxidant defence system (Nrf2, PPAR-γ) in the LHV.

In conclusion, the results showed tissue-dependent effects of MLN in SHR rats. In the liver, 14-day administration of MLN increased the NOS activity but did not alter the mRNA expression of eNOS, iNOS and nNOS and other genes investigated in this study. In LHV, the NOS activity and superoxide production were not altered after MLN administration, but we observed significant genomic effects. These may suggest delayed influence of MLN in the cardiac tissue which may be manifested by later onset of cardiac dysfunction.

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THE CONSEQUENCES OF MLN-4760 AND ZOFENOPRIL ADMINISTRATION ON MMP ACTIVITIES AND OXIDATIVE STRESS IN SPONTANEOUSLY HYPERTENSIVE RATS

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Hypertension is the most prevalent risk factor in patients suffering from COVID-19. Inflammation and increased oxidative stress accompany hypertension and its etiopathogenesis. Matrix metalloproteinases (MMPs) are proteolytic enzymes with multiple functions, that are involved in the pathogenesis of numerous diseases. MMP-2 is produced by fibroblasts, endothelial cells, and osteoblasts and is involved mainly in physiological remodelling of tissue. MMP-9 is produced mainly by activated immune cells, mostly macrophages. In hypertensive patients, the inhibition of the angiotensin-converting enzyme by enalapril lowered MMP-9 activities. The plasma levels of MMP-9 positively correlated with the risk of death in patients suffering from COVID-19. The aim of the present study was to investigate the effect of ACE2 inhibition and zofenopril administration on MMP-2 and -9 activity under monitoring selected parameters of oxidative stress and antioxidative status in spontaneously hypertensive rats (SHR).
ACE2 inhibition was induced by MLN-4760 (1 mg/kg/day for 2 weeks). Zofenopril administration (10 mg/kg/day) started after 4-days of ACE2 inhibition. Experimental animals were divided into 4 groups: control (C, n = 12), control zofenopril-treated (CZ, n = 7), MLN-4760-treated (M, n = 12), and MLN-4760 and zofenopril-treated group (MZ, n = 12). Activities of MMP-2 and MMP-9 were detected by gelatine zymography in plasma samples. The equilibrium levels of angiotensin (Ang I - IV) were quantified by liquid chromatography mass spectrometry/mass-spectroscopy in plasma samples. Markers of oxidative stress (GSH/GSSG ratio), antioxidant status (FRAP), total antioxidant capacity (TAC), carbonyl stress (AGEs), protein oxidation (AOPP), and lipid peroxidation (TBARS) were determined in plasma samples and also in hemolyzed red blood cells (RBCs) using spectrophotometric and fluorometric methods.
In plasma samples, MZ rats had higher TAC compared with M group ($p < 0.05$). GSH/GSSG ratio was significantly higher in M compared with C ($p < 0.05$) and MZ ($p < 0.01$). In hemolyzed RBCs, zofenopril treatment decreased GSH/GSSG ratio in control rats. This ratio was also lower in M compared with C. Concentration of FRAP was higher in M compared with C, and decreased after zofenopril treatment in ACE2 inhibited rats. MMP-9 activity in plasma was unchanged after ACE2 inhibition ($p = 0.21$, C versus M). Zofenopril administration increased plasma MMP-9 activities in controls ($p < 0.001$, C versus CZ group), as well as in ACE2-inhibited ($p < 0.001$, M versus MZ group) rats. No significant differences were detected in MMP-2 activities among the groups. According to two-way ANOVA analysis, MLN administration increased Ang II concentration in plasma. Zofenopril treatment increased plasma concentration of each angiotensin - Ang I, Ang II, Ang 1-5 and Ang 1-7 in control rats and ACE2-inhibited rats.
ACE2 inhibition increased GSH/GSSG ratio of SHR. It may be the consequence of systemic adaptive responses to ACE2 inhibition in this model of genetic hypertension. However, GSH/GSSG ratio was lower and FRAP higher in RBCs in M group compared with control animals. This suggests deterioration of oxidative balance in the intracellular compartment following the ACE2 inhibition in SHR model of hypertension. The increase in Ang II concentration after zofenopril treatment may be, at least partially, related to increase in MMP-9 activity.

N-ACETYLCYSTEINE IN MECHANICALLY VENTILATED RATS WITH LIPOPOLYSACCHARIDE-INDUCED ACUTE RESPIRATORY DISTRESS SYNDROME: THE EFFECT OF INTRAVENOUS DOSE ON OXIDATIVE DAMAGE AND INFLAMMATION

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Background: Acute respiratory distress syndrome (ARDS) is a common cause of respiratory failure and is associated with high morbidity and mortality. ARDS is multifactorial syndrome and it is often modelled by lipopolysaccharide (LPS) administration in the lungs. The search for suitable therapy in ARDS patients is still going on. There are only few animal studies regarding the use of N-acetylcysteine (NAC) in ARDS. To date, the effect of NAC has only been evaluated in spontaneously breathing animals. Aim: To evaluate the effect of two different intravenous (i.v.) doses of NAC on oxidative stress, inflammation and lung functions in recently developed model of severe LPS-induced ARDS requiring mechanical ventilation and oxygen treatment, as this model reflects the situation of the patients at intensive care units more realistically.
Methods: Adult Wistar rats were intratracheally instilled with LPS (500 μg/kg; 2.2 ml/kg) or saline (controls) and mechanically ventilated with tidal volume 6 mL/kg, positive end-expiratory pressure 0.3 kPa, inspiration time 50%, 40% oxygen and respiratory rate 60 breaths per minute. Animals with LPS were further treated with i.v. NAC 10 mg/kg b.w. (NAC10) or 20 mg/kg b.w. (NAC20). Lung functions (PaO_2/FiO_2; ventilation efficiency index, VEI; oxygenation index, OI; and alveolar-arterial gradient, AaG) were evaluated every hour. After the experiment, left lung was lavaged, right lung was homogenized for evaluation of local and systemic oxidative and inflammatory changes. Lung oedema was expressed as wet/dry lung weight ratio. White blood cell (WBC) count and neutrophil count were evaluated in arterial blood and bronchoalveolar lavage fluid.
Results: In comparison to control, LPS increased lung oedema formation, oxidative stress, levels of inflammatory markers, had negative impact on the lung functions and caused the shift in WBC between the blood and lavage fluid. NAC significantly improved PaO_2/FiO_2, AaG, VEI and OI and reduced WBC content in the lungs and reduced lung oedema, oxidative stress and inflammation in the lungs. NAC20 in comparison to NAC10 was more effective in reduction oxidative damage of lipids and proteins, and some of inflammatory markers (IL-6, GM-CSF) and ICAM-1.
Conclusions: LPS-instilled and mechanically ventilated rats may be a suitable model of ARDS to test the acute effects of therapies at organ, systemic, cellular and molecular levels. I.v. NAC, in rather low dose, improves lung functions, reduces neutrophil migration into the lung, and reduces lung oedema, oxidative stress and wide range of pro-inflammatory mediators. Higher dose of NAC is more powerful as for reduction of oxidative damage and inflammation almost to baseline. The results together with literary data support the potential of NAC in ARDS.
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ASSESSMENT OF PLATELET MITOCHONDRIAL RESPIRATION AND THE ROLE OF PERMEABLE SUCCINATE IN CHILDREN DIAGNOSED WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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Mitochondrial dysfunction in peripheral blood cells has recently emerged as potential biomarker in the evaluation of various non-communicable diseases such as diabetes mellitus, cancer, cardiovascular and neurodegenerative diseases. A recent study revealed that platelet mitochondrial dysfunction was present in adult patients diagnosed with hematological malignancies who were undergoing chemotherapy. Whether platelet mitochondrial dysfunction is present at the onset of hematological malignancies it is not known.
The present pilot study performed in a pediatric population was aimed to assess: a) the changes in platelet mitochondrial respiration at the onset of acute lymphoblastic leukemia (ALL) and b) the effect of a novel compound, permeable succinate, on platelet respiration. Platelet isolation was performed by differential centrifugations at room temperature. Platelet mitochondrial respiration was assessed at 37°C by means of high-resolution respirometry (Oxigraph-2k, Oroboros Instr., AT) according to a classical Substrate-Uncoupler-Inhibitor-Titration (SUIT) protocol. Platelets were suspended in a respiration medium (MIRO5) and permealized with digitonin. After obtaining the routine state, complex I substrates (glutamate and malate) and ADP were added in order to measure the active respiration or OXHPOS capacity dependent on complex I (OXPHOS C I). Afterwards, succinate, a complex II substrate, was added to determine the maximal active respiration (OXPHOS capacity). In order to assess the non-phosphorylating respiration (LEAK), oligomycin, an ATP synthase inhibitor, was added. A classical uncoupler, FCCP (carbonyl cyanide p-trifluoro-methoxy phenyl-hydrazone) was titrated in successive steps to measure the maximal non-coupled respiration or the ET capacity. For the measurement of the ET capacity dependent only on complex II, rotenone a complex I inhibitor, was added. In this pilot study, a significant increase in complex I-supported active respiration and a decrease in maximal noncoupled respiration were found at the disease onset. Second, we assessed platelet mitochondrial respiration in the presence of a novel compound, a succinate prodrug in patients with ALL in remission. An increase in all respiratory parameters but particularly in the ET capacity, was found.
In conclusion, our preliminary results firstly revealed: a) the occurrence of mitochondrial dysfunction at the onset of pediatric ALL and b) the beneficial role of permeable succinate.

INHIBITION OF SOLUBLE EPOXIDE HYDROLASE ATTENUATES BOSUTINIB-INDUCED BLOOD PRESSURE ELEVATION

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Endothelial cells (ECs) play a critical role in maintaining homeostasis of vascular function, and endothelial activation is involved in the initial step of atherogenesis. Previously, we reported that Abl kinase mediates shear stress-induced endothelial activation. Bosutinib, a dual inhibitor of Src and Abl kinases, exerts an atheroprotective effect; however, recent studies have demonstrated an increase in the incidence of side effects associated with bosutinib, including increased arterial blood pressure (BP). To understand the effects of bosutinib on BP regulation and the mechanistic basis for novel treatment strategies against vascular dysfunction, we generated a line of mice conditionally lacking c-Abl in ECs (EC-Abl^{KO}). Knockout mice and their wild-type littermates (Abl$^{f/f}$) were orally administered a clinical dose of bosutinib, and their BP was monitored. Bosutinib treatment increased BP in both EC-Abl^{KO} and Abl$^{f/f}$ mice. Furthermore, acetylcholine-evoked endothelium-dependent relaxation of the mesenteric arteries was impaired by bosutinib treatment. RNA sequencing of mesenteric arteries revealed that the cytochrome P450 (CYP)-dependent metabolic pathway was involved in regulating BP after bosutinib treatment. Additionally, bosutinib treatment led to an upregulation of soluble epoxide hydrolase (sEH) in the arteries and a lower plasma content of eicosanoid metabolites in the CYP pathway in mice. Treatment with 1-Trifluoromethoxyphenyl-3-(1-propionylpiperidin-4-yl) urea (TPPU), a sEH inhibitor, reversed the bosutinib-induced changes to the eicosanoid metabolite profile, endothelium-dependent vasorelaxation, and BP. Thus, the present study demonstrates that upregulation of sEH mediates bosutinib-induced elevation of BP, independent of c-Abl. The addition of sEH inhibitor in patients treated with bosutinib may aid in preventing vascular side effects.

THE EFFECT OF (±)-TAXIFOLIN ON BEHAVIOUR AND OXIDATIVE STATE IN SPONTANEOUSLY HYPERTENSIVE RATS TREATED WITH MLN-4760, AN INHIBITOR OF ANGIOTENSIN CONVERTING ENZYME 2

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SARS-CoV-2 virus, which causes COVID-19, enters the cells via binding to angiotensin converting enzyme 2 (ACE2) on cellular membrane, which results in severe acute respiratory syndrome. It may have also potential negative consequences on the cardiovascular health, namely in subjects with pre-existing cardiovascular diseases, including hypertension. In computational studies, (−)-taxifolin (TX) was recognized as a possible inhibitor of SARS-CoV-2 main protease M^{pro}, which suggested that TX could reduce virus replication. Thus the aim of this study was to investigate the effects of TX in SHR rats, in which MLN-4760, an ACE2 inhibitor, was infused subcutaneously to mimics the action of SARS-CoV-2. We investigated the effects of (±)-TX in MLN-treated rats on blood pressure (BP), heart rate (HR), body weight (BW) and spontaneous behaviour in the open field test. We also investigated antioxidant effects of TX and gene expressions of selected antioxidant genes in left heart ventricle and brainstem. MLN was infused subcutaneously, diluted in 10% DMSO at the dose of 1 mg/kg/day for 14 days, by means of Alzet osmotic minipumps. TX was administered from day 5 of experiment diluted in drinking water at the dose 20 mg/kg/day.

Taxifolin had no effects on BP and HR in MLN-treated rats. However, TX reduced relative increase in body weight compared to MLN-treated group. There were no alterations in total distance, time immobile, central time and relative central distance travelled in open field test, showing no effect of TX on spontaneous behavior and anxiety-like behavior of SHR. However, TX significantly decreased superoxide production and elevated the expressions of superoxide dismutase 1 and 2 (SOD1, SOD2), nuclear factor erythroid 2-related factor 2 (NRF2), heme oxygenase 1 (HO-1), glutathione peroxidase 4 (Gpx4) and peroxisome proliferator-activated receptor gamma (PPAR γ) mRNA in the left heart ventricle. On the other hand, there were no changes in mRNA expressions of SOD 1, SOD2, NRF2, HO-1, Gpx4 and PPAR γ genes in the brain stem.

In conclusion, TX does not affect spontaneous behavior of rats and antioxidant gene expressions in the brainstem, suggesting that TX (or its active metabolites) may not cross blood-brain-barrier and/or to produce significant antioxidant effects in the brain. On the other hand, significant antioxidant effects of TX in the heart may provide cardioprotective effects in during COVID-19.

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ARTERIAL STIFFNESS IN HYPERTENSION

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Arterial stiffness is an index of vascular aging and damage, correlated with long-term cardiovascular risk. It is still not clear if an increased arterial stiffness is the cause or consequence of hypertension. Elastin fiber damage, fragmentation, and loss, and increased production and accumulation of collagen within the arterial wall of the large arteries, enable the transmission of potentially harmful pulsatile energy into the microcirculation causing target organ damage. Several biomarkers were correlated with pulse wave velocity and early vascular aging in hypertensive patients, including electrocardiographic and serological variables. Further long-term studies are needed to provide insights related to the crosstalk between arterial stiffness and hypertension.

THE ROLE OF MONOAMINE OXIDASE IN CARDIOMETABOLIC DISEASES: AN ONGOING SAGA

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With the ageing of the population, the global burden of cardiometabolic diseases (cardiovascular disease, obesity and diabetes) is steadily rising. Mortality due to cardiovascular diseases has declined in the past decades in high-income countries but increased in low- and middle-income countries; also, the prevalence of diabetes has increased worldwide but at a faster rate in the latter group. The major pathomechanisms that underlie all these pathologies are chronic oxidative stress and low-grade inflammation that promote each other in a vicious circle leading to both disease progression and the occurrence of complications. Monoamine oxidase (MAO) with two isoforms (A and B) are flavoenzymes located at the outer mitochondrial membrane that have been reported to contribute to the oxidative stress in cardiovascular system and adipose tissue. While ageing has been systematically associated with a higher MAO activity/expression, chronic inflammation is responsible for an age-independent increase in MAO expression. MAO-A is overexpressed in both visceral adipose tissue and vascular system and is responsible for endothelial dysfunction in the setting of obesity associated with inflammatory status, whereas MAO-B isoform is mainly increased in vessels of diabetic animals and humans. MAO inhibition significantly alleviated oxidative stress suggesting that MAO inhibitors are promising candidates for drug repurposing.

RESISTANCE EXERCISE-INDUCED CARDIAC REMODELING

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Hypertrophic remodeling serves as a reactive mechanism to compensate for the heavy load during training. Resistance exercises lead to an increase in systolic and diastolic blood pressure, which results in concentric hypertrophy, while endurance training (long-term running, for example) is characterized by an increase in left ventricular dilatation and the development of eccentric hypertrophy. The type of resistance training (eg, Olympic weightlifting, powerlifting, bodybuilding, specific fitness resistance training) may mediate the extent of changes in left ventricular morphology due to differences in training volume and intensity. In powerlifting, training is based on the development of maximum strength and in specific heavyweight exercises (squat, bench press, and deadlift) during one to eight maximum repetitions. On the other hand, specific fitness resistance training in athletes usually include high repetitions with lightweight. The impact of various forms of resistance training on cardiac morphology and function in females have not been comprehensively investigated, which often raises doubts about the changes that occur, both in professional athletes and females who are just starting to exercise. Hypertrophic response in females are considered a physiological adaptation to resistance training and structural cardiac remodeling may occur, without impairment of cardiac function. Echocardiographic screening should be considered when discriminating the athlete's heart from potential cardiovascular dysfunction, because both remodeling, physiological and pathological, have a lot of similarities in the early phase. Powerlifting, resistance training, the use of dumbbells, barbell weights, and various exercises equipment is not reserved only for men. On the contrary, females should not avoid heavy weight exercises, when the resistance training is properly planned and programmed.

Key words: Cardiac remodeling, Resistance training, Echocardiography, Physiological adaptation.

MONOAMINE OXIDASE AND METABOLIC MEMORY: REPOSITIONING MAO INHIBITORS AS POTENTIAL VASCULOPROTECTIVE AGENTS IN EXPERIMENTAL DIABETES

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Monoamine oxidases (MAOs) are mitochondrial dehydrogenases with two isoforms, A and B, which catalyze at the outer mitochondrial membrane the electron transfer from biogenic amines to molecular oxygen via a reaction that generates hydrogen peroxide (H_2O_2) as by-product. We have previously reported an increased aortic expression of both MAO isoforms in murine models associated with vascular inflammation (induced by lipopolysaccharide) and hypertension (after angiotensin II administration via minipumps) which was responsible for increased reactive oxygen species (ROS) production and subsequent endothelial dysfunction.
The aim of the present study was to assess the contribution of MAOs to the "hyperglycemic or metabolic memory" phenomenon, as potential mechanism underlying the persistent oxidative damage and endothelial dysfunction in an experimental model of murine diabetes mellitus. We hypothesized that: i) MAOs become a crucial contributor to vascular oxidative stress and dysfunction, in the setting of experimental diabetes, even after glucose normalisation and ii) selective MAO-A and MAO-B inhibition (with clorgyline and selegiline, 1 mg/kg/day, 1 week, respectively) in the vasculature will mitigate the deleterious effects of MAO-related oxidative stress. Our results showed that: i) MAOs expression increased in mice aortas (assessed by qRT-PCR and immune fluorescence) after 2 weeks of hyperglycemia together with high ROS generation (assessed in spectophotometry and confocal microscopy - DHE staining) and impaired vascular relaxation (myograph studies), ii) glucose normalization (glargine, 10 U/kg/day, 1 week) failed to significantly reduce MAO-derived ROS generation and improve vascular function, and iii) in vivo administration of MAO inhibitors (in addition to insulin) reduced ROS levels and alleviated endothelial dysfunction in diabetic mice. In conclusion, MAOs represent a potential therapeutic target in the diabetic vessels that significantly contribute to endothelial dysfunction. MAO inhibitors blunted the vascular "hyperglycemic memory" and are viable candidates for drug repurposing as vasculo-protective agents in diabetes.
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INVESTIGATION OF FREE RADICAL SCAVENGING CAPACITY OF TAXIFOLIN, ZOFENOPRIL AND MLN-4760

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High-molar-mass hyaluronan (HA) is a glycosaminoglycan present in various tissues, especially in skin and synovial joints. The aim of the study was to explore potential protective effects of taxifolin and MLN-4760 against reactive oxygen species formed by Cu(II) ions and ascorbate, which induced degradation of high-molar-mass HA [1]. Further, we assessed radical scavenging capacities of taxifolin, zofenopril and MLN-4760 by standard ABTS and DPPH assays. The results showed that taxifolin inhibited HA degradation either by OH radicals or alkoxy-/peroxy-type radicals. On the other hand, MLN-4760 did not inhibit HA degradation. Unlike zofenopril and MLN-4760 high radical scavenging capacity of taxifolin was shown when assessed by the ABTS and DPPH assays.

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THE ACTIVITY OF NITRIC OXIDE SYNTHASE IN THE RAT BRAIN DEPENDS ON THE DURATION OF SOCIAL ISOLATION

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Social isolation deprives rodents of social interactions that are critical for normal development of brain and behavior. Several studies have indicated that postweaning isolation rearing may affect nitric oxide (NO) production. The aim of this study was to compare selected behavioral and biochemical changes related to NO production in the brain of rats reared in social isolation for different duration. At the age of 21 days, male Sprague Dawley rats were randomly assigned into four groups reared in isolation or socially for 10 or 29 weeks. At the end of the rearing, open-field and prepulse inhibition (PPI) tests were carried out. Furthermore, in several brain areas we assessed NO synthase (NOS) activity, protein expression of nNOS and iNOS isoforms and the concentration of conjugated dienes (CD), a marker of oxidative damage and lipid peroxidation. Social isolation for 10 weeks resulted in a significant decrease in PPI, which was accompanied by a decrease in NOS activity in the cerebral cortex and the cerebellum, an increase in iNOS in the hippocampus and an increase in CD concentration in cortex homogenate. On the other hand, a 29 week isolation had an opposite effect on NOS activity, which increased in the cerebral cortex and the cerebellum in animals reared in social isolation, accompanied by a decrease in CD concentration. The decrease in NOS activity after 10 weeks of isolation might have been caused by chronic stress induced by social isolation, which has been documented in previous studies. The increased oxidative state might result in the depleted NO bioavailability, as NO reacts with superoxide radical creating peroxynitrite. After 29 weeks of isolation, this loss of NO might be compensated by the subsequent increase in NOS activity.

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Keywords: social isolation; neurodevelopment; nitric oxide; nitric oxide synthase; oxidative stress

MOLECULAR MECHANISM OF PAFAH1B2 ON ATHEROSCLEROSIS FORMATION

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Background: Atherosclerosis is a chronic inflammatory disease. Studies have shown that the NLRP3 inflammatory complex is involved in vascular inflammation and atherosclerosis, and caspase-1 plays an important role in the development of atherosclerosis as an effector protein of NLRP3.
Methods and results: We screened and identified the gene PAFAH1B2 regulated by caspase-1 using whole-genome microarray analysis in capsase-1 knock-down human umbilical vein endothelial cells (HUVEC). Using adenovirus to knock down PAFAH1B2 in HUVEC decreased the expression of NF-κB, NLRP3, caspase-1 and IL-1β in the level of mRNA and proteins, which indicated that inhibition of PAFAH1B2 can attenuate excessive inflammatory responses by suppressing the IL-1β expression. After tail vein injection of adeno-associated virus interfering with PAFAH1B2, 8-10 week-old male ApoE-/- mice were fed with high fat for 16 weeks. The results of oil red O staining of mouse aorta and aortic root showed that the aortic plaque was significantly reduced when PAFAH1B2 expression was decreased ($P<0.05$). Plasma LDL was reduced by about 11% ($P=0.0483$) and IL-1β was reduced by about 42% ($P=0.0041$), compared with the control group.
Conclusions: This study suggests that inhibition of PAFAH1B2 expression may inhibit inflammation by reducing IL-1β expression through NF-κB/NLRP3/caspase-1 signaling pathway, thereby inhibiting the development of atherosclerosis.

NEURONAL APOE4 STIMULATES C/EBPB ACTIVATION, PROMOTING ALZHEIMER'S DISEASE PATHOLOGY IN A MOUSE MODEL

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ApoE4 is a major genetic risk determinant for Alzheimer's disease (AD) and drives its pathogenesis via Aβ-dependent and -independent pathways. C/EBPβ, a proinflammatory cytokine-activated transcription factor, is upregulated in AD patients and increases cytokines and □-secretase expression. Under physiological conditions, ApoE is mainly expressed in glial cells, but its neuronal expression is highly elevated under pathological stresses. However, how neuronal ApoE4 mediates AD pathologies remains incompletely understood. Here we show that ApoE4 activates C/EBPβ that subsequently regulates APP, Tau and BACE1 mRNA expression in mouse neurons, driving AD-like pathogenesis. To interrogate the pathological roles of both human ApoE4 and C/EBP□ elevation in neurons in the aged brain, we develop neuronal specific Thy1-ApoE4/C/EBPβ double transgenic mice. Neuronal ApoE4 strongly activates C/EBPβ and augmented □-secretase subsequently cleaves increased mouse APP and Tau, promoting AD-like pathologies. Notably, Thy1-ApoE4/C/EBPβ mice develop amyloid deposits, Tau aggregates and neurodegeneration in an age-dependent manner, leading to synaptic dysfunction and cognitive disorders. Thus, our findings demonstrate that neuronal ApoE4 triggers AD pathogenesis via activating the crucial regulator C/EBPβ. Neuronal ApoE4-activated C/EBP□/□-secretase pathway might underlie the key dominant pathologic mechanism driving AD pathogenesis. Clearly, this powerful sporadic mouse model will provide an unprecedented research tool for analyzing biological functions on APP and Tau and many other AD players and pathological evaluation of the truncated products in AD onset and progression. Conceivably, this mouse model will facilitate the desperate AD drug development.

Keywords: Alzheimer's Disease; transcription factor; C/EBPβ; ApoE4; animal model

TARGETING INFLAMATORY PARACRINE NETWORK VIA INHIBITING GALECTIN-3 ALLEVIATES ACUTE SYMPATHETIC ACTIVATION-INDUCED CARDIAC INJURY

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Inflammation plays an important role in cardiovascular diseases. Following acute sympathetic stress, cardiac inflammation is initiated via activation of the inflammasome and the downstream interleukin-18 (IL-18). However, IL-18 blockage only suppress the inflammation at early phase, which limits its clinical application. The present study aims to clarify the key mechanism in cardiac inflammatory initiated by IL-18 and to identify new anti-inflammatory targets with a wider therapeutic window for treatment of acute sympathetic stress-induced cardiac injury.

Methods and results

We used bioinformatics analysis and screened out galectin-3 as the potential downstream molecule of IL-18. In the wild-type mice, the galectin-3 expression was upregulated in hearts after IL-18 activation following isoproterenol (ISO, 5 $mg \cdot kg^{-1}$) treatment. A positive correlation was observed between the plasma levels of galectin-3 and norepinephrine or IL-18 in patients with chest pain. The myocardial galectin-3 upregulation was attenuated in $Nlrp3^{-/-}$ and $Il18^{-/-}$ mice. ISO treatment increased galectin-3 expression only in the macrophages cocultured with cardiomyocytes, but not macrophages or cardiomyocytes themselves. ISO-induced expression of galectin-3 in macrophages was decreased by blocking IL-18 with neutralizing antibody or $Il18^{-/-}$ cardiomyocytes. Deletion of galectin-3 gene suppressed ISO-induced cardiac inflammatory injuries. Moreover, treatment with galectin-3 inhibitor, but not a β-blocker, one day after ISO treatment effectively attenuated cardiac inflammatory injuries and improved cardiac function.

Conclusion

IL-18/galectin-3 axis mediates the cardiac inflammatory injuries induced by acute β-AR activation. Galectin-3 inhibitor, but not a β-blocker, treatment one day after β-AR insult can successfully block cardiac inflammatory injuries upon acute β-AR overactivation.

ENDOTHELIAL TRANSCRIPTION FACTOR EB ATTENUATES VASCULAR INFLAMMATION OF DIABETIC DB/DB MICE THROUGH RESTRAINING IKK (IKB KINASE)-P65 PATHWAY

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Background: Transcription factor EB (TFEB) is the master regulator of autophagy and lysosomal biogenesis. Recently, TFEB was shown to be induced by atheroprotective laminar flow and play an anti-atherosclerotic effect through inhibiting endothelial inflammation and oxidative stress. However, the mechanism remain largely unclear.
Objective: To explore whether TFEB regulates endothelial inflammation in diabetic db/db mice and its potential mechanisms.
Methods and Results: Western blotting results showed that TFEB was mainly expressed in endothelial cells (ECs) in mouse aortas. Compared with db/m+, db/db mouse aortas showed increased p-TFEB (S142) and decreased total TFEB protein level, suggesting a decrease in TFEB expression and activity. Likewise, the total TFEB protein level in the pulmonary ECs of db/db mice was significantly lower than that of db/m+. As diabetes risk factors, interleukin-1β and insulin significantly increased the phosphorylation level of TFEB in human umbilical vein ECs (HUVECs). In vivo and ex vivo overexpressing TFEB attenuated db/db mouse aortic endothelium inflammation, as evidenced by decreased expression of vascular inflammatory markers and adhesion molecules. In vitro overexpressing TFEB suppressed HUVECs inflammation and adhesion with THP1 monocyte. RNA sequencing result showed that nuclear factor-kappa B (NF-κB) signaling was inhibited after TFEB overexpression in HUVECs. Indeed, luciferase assay showed suppressed NF-κB transcriptional activity by TFEB. Mechanistically, TFEB reduced p65 nuclear translocation by inhibiting IκB-α kinase (IKK) phosphorylation and subsequent IκB-α degradation. PS-1145, an IKK inhibitor, abolished the inflammatory state of HUVECs after TFEB silencing. In addition, laminar flow-induced TFEB expression through Krüppel-like factor 2 (KLF2). TFEB overexpression reversed shKLF2-induced upregulation of vascular cell adhesion protein 1 in HUVECs when exposing to laminar flow.
Conclusions: This study mainly proves that TFEB exerts anti-inflammatory effect in diabetic mouse ECs by inhibiting the canonical IKK-NF-κB pathway. Laminar flow induces TFEB expression and reduces inflammation through KLF2.
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SILICONE PROSTHETICS AND AUTOIMMUNE PATHOLOGY OF THYROID

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The medical community's views on plastic surgery rooms with silicone implants range diametrically from the "harmless" [1] to “unsafe” [2]. The latter point of view seems to be more plausible if we take into account the large-scale epidemiological studies of Coroneos et al., showed a significantly increased risk of developing autoimmune pathology in carriers of silicone implants [3]. Taking into account that the most common nosological form in autoimmunology is the autoimmune pathology of the thyroid gland in general, and autoimmune thyroiditis in particular, it is of interest to study the role of silicone not only as a trigger of the autoimmune process in general, but the autoimmune process in particular in the thyroid gland.

Aim: The aim of this work is to assess the effect of silicone breast implants (SBI) on the autoimmune and endocrine status in silicone mammoplasty.

Research objectives: We analyzed the outcomes of 119 aesthetic surgical corrections of the shape and volume of the mammary gland (MG), as well as therapeutic and reconstructive interventions on the MG in 106 patients before, 3, 6 and 12 months after surgery. The average age of the operated patients was 39.2 ± 11.4 years. Patients were included in the study and underwent surgical treatment from September 2018 to November 2019, followed by follow-up up to a year after surgery. During the observation period, a total of 119 operations were performed on 106 patients 79 patients done one - year follow up. Studying of the immune status: determination of autoantibodies to modified citrullinated vimentin (MCV-Ab), cardiolipin (ACLA IgG and ACLA IgM), beta2-glycoprotein 1 (Anti-β2-GP1), thyrotropic hormone receptor (TSHR-Ab), thyroglobulin (TG-Ab), annexin V (aAnV IgG and aAnV IgM) before surgery and at all control periods.Studying of the hormonal status: determination of the content of thyrotropin (TSH), prolactin (PRL), estradiol (E), testosterone (TC), triiodothyronine (T3) before surgery and at all control periods.

Results: Almost in one half of patients in the point 0 (before surgery), prolactin level was considerably higher compared to the age norm. In dynamics after the surgery, prolactin level in patients decreased to normal range and remained within it during all 12 months after the operation. 78.1% of our patients had a significant pathological level of autoantibodies to the thyrotropic hormone receptor a year after the installation of silicone implants. In our study, the increased antibody levels against thyrotropin receptors, significantly different from the controls, was not accompanied by an increase in the levels of thyroid hormones in patients. Based on the results, the following should be recommended: refused surgery using silicone implants in patients who has antibodies to the thyrotropic hormone receptor in the blood serum or von Basedow-Graves disease and needed need long-term follow-up with mandatory control of the level of antibodies to the thyrotropic hormone receptor.

Conclusion: Silicone mammoplasty in some cases is accompanied by the adjuvant action of silicone, and in 78.1% of cases it is manifested by the year of observation by an excess (more than 2 times) of the level of autoantibodies to the thyrotropic hormone receptor (TSHR-Ab).

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