


18th Annual symposium of Croatian Physiological Society

with international participation

17th – 18th October 2025

Croatian Institute for Brain Research

University of Zagreb School of Medicine

ZAGREB, 2025

PUBLISHED BY

Croatian Physiological Society

FOR PUBLISHER

Prof. Aleksandra Dugandžić, MD, PhD

EDITOR

Asst. Prof. Nikola Habek, MD, PhD

DESIGN

Asst. Prof. Nikola Habek, MD, PhD

VENUE

Croatian Institute for Brain Research
University of Zagreb School of Medicine

GENERAL INFORMATION

SCIENTIFIC COMMITTEE

Aleksandra Dugandžić, Ines Drenjančević, Ines Mrakovčić-Šutić, Željko Dujić, Lana Ružić, Ana Stupin, Hrvoje Lalić, Ana Shek-Vugrovečki, Ivana Šutić, Nikola Habek, Helena Lenasi, Naim Akhtar Khan, Markus Ritter

ORGANIZING COMMITTEE

Nikola Habek, Martina Ratko, Anja Barić

LOCAL ORGANIZER

Department of Physiology
Department of Neuroscience
Croatian Institute for Brain Research
University of Zagreb School of Medicine
Šalata 3, 10000 Zagreb, Croatia

SPONSORS

SCIENTIFIC PROGRAMME OVERVIEW

KEYNOTE LECTURES

Naim Akhtar Khan (Centre for Translational and Molecular medicine, Physiologie de la Nutrition & Toxicologie, France)

Markus Ritter (Institute for Physiology and Pathophysiology, Salzburg, Austria)

INVITED SPEAKERS

Nina Vardjan (University of Ljubljana, Faculty of Medicine)

Ivana Jarić (Institute of Laboratory Animal Science, University of Zürich, Switzerland)

Aleksandra Dugandžić (School of Medicine, University of Zagreb)

Marina Dobrivojević Radmilović (School of Medicine, University of Zagreb)

Ines Drenjančević (Faculty of Medicine of University of Osijek)

Darja Flegar (School of Medicine, University of Zagreb)

Ines Mrakovčić-Šutić (The Faculty of Medicine, University of Rijeka)

Hrvoje Lalić (School of Medicine, University of Zagreb)

Helena Lenasi (University of Ljubljana, Faculty of Medicine)

PROGRAMME

Friday 17th October 2025

10.00 – 11.00	**REGISTRATION AND COFFEE**
11.00 – 11.45	**KEYNOTE LECTURE** **Naim Akhtar Khan:** Obesity: a matter of fat taste
11.45 – 12.15	**Invited Lecture** **Nina Vardjan:** Dysregulation of Signalling and Metabolism in the Aging Drosophila Brain
12.15 – 12.30	**COFFEE BREAK**
12.30 – 13.50	**Session 1. Metabolism: from physiology to clinics** Chairs: Aleksandra Dugandžić, Marina Dobrivojević Radmilović
12.30 – 13.00	**Ivana Jarić:** Beyond SABV: Sex Steroid Hormone Status as a Key Factor in Detecting Sex Differences
13.00 – 13.25	**Aleksandra Dugandžić:** Expression of guanylate cyclase C and uroguanylin in mouse and human brain depend on sex/gender, phase of estrous cycle, age and feeding status
13.25 – 13.50	**Marina Dobrivojević Radmilović:** Bradykinin type 2 receptor deficiency alters vascular endothelial growth factor and atrial natriuretic peptide levels in early-stage diabetic retinopathy
14.00 – 15.30	**LUNCH BREAK:** Student canteen of the School of Medicine
15.00 – 15.30	Closed meeting of Executive Committee
15.30 – 16.30	**CPS (HDF) Assembly** – all members of CPS
16.30 – 18.00	**POSTER SESSION** (PP presentations)
18.00	Taking photo
19.00	CONGRESS DINNER

Saturday 18th October 2025

09.30 – 10.00	**COFFEE**
10.00 – 10.45	**KEYNOTE LECTURE** **Markus Ritter:** Hydration and volume homeostasis - from cells to body and population
10.45 – 11.45	**Session 2: Patophysiology 1** Chairs: Ines Drenjančević, Darja Flegar
10.45 – 11.10	**Ines Drenjančević:** Endothelial glycocalix- a barrier or a mediator?
11.10 – 11.35	**Darja Flegar:** The role of gut microbiota in circadian rhythm of bile acid levels
11.35 – 11.45	*Short talk:* **Vedrana Tudor Špalj**: Association between pulse wave velocity and cardiovascular risk factors in patients with spondyloarthritis
11.45 – 12.00	**COFFEE BREAK**
12.00 – 13.45	**Session 2: Patophysiology 2** Chairs: Ana Stupin, Hrvoje Lalić
12.00 – 12.25	**Ines Mrakovčić-Šutić:** Granulysin and perforin mediated cytotoxicity in patients with colorectal cancer
12.25 – 12.50	**Hrvoje Lalić:** Decoding chronic graft-versus-host disease with monocyte signatures and plasma chemokines
12.50 – 13.15	**Helena Lenasi:** Acute hyperglycemia and (micro)vascular function: impact of experimental model, vessel size, sex and methodological approach
13.15 – 13.30	*Short talk:* **Klara Bardač:** Metabolic impact of stearoyl-coenzyme A desaturase inhibition on acute myeloid leukemia cells
13.30 – 13.45	*Short talk:* **Željka Minić** (Faculty of Biotechnology and Drug Development, University of Rijeka): Central control of sympathetic nerve activity in a rat: the role of spinal cord and forebrain centers
13.45	CLOSING CEREMONY AND AWARDS

ABSTRACTS

INVITED LECTURES:

Expression of guanylate cyclase C and uroguanylin in mouse and human brain depend on sex/gender, phase of estrous cycle, age and feeding status

Nikola Habek[1-3], Martina Ratko[1,2], Vladiana Crljen[1-3], Martina Tkalčić[4], Anton Mažuranić[4], Pero Bubalo[4], Petar Škavić[4], Aleksandra Dugandžić[1-3]

[1] Croatian Institute for Brain Research, School of Medicine, University of Zagreb, Zagreb, Croatia;
[2] Centre of Excellence for Basic, Clinical and Translational Neuroscience, School of Medicine, University of Zagreb, Zagreb, Croatia;
[3] Department of Physiology, School of Medicine, University of Zagreb, Zagreb, Croatia;
[4] Institute for Forensic Medicine, School of Medicine, University of Zagreb, Zagreb, Croatia

Brain uroguanylin (UGN) play a role in the regulation of glucose metabolism and body weight by activation of brown adipose tissue and satiety. Observed gender differences in UGN brain function may occur due to difference in expression of UGN receptor (guanylate cyclase C (GC-C)) or the hormone precursor proUGN. In this study, we determined GC-C and proUGN expressions in the prefrontal and cerebellar cortex, arcuate nucleus of hypothalamus and substantia nigra by ELISA, immunohistochemistry and in situ hybridisation. Study was performed on 21 male (10 subjects with obesity) and 13 female brains (6 subjects with obesity) with postmortem delay less than 24 h. In the human prefrontal cortex, proUGN was expressed in male BA9 and 10 was downregulated if subjects died shortly after a meal. In male subjects with obesity, proUGN expression in BA10 was not regulated by feeding. Furthermore, in hypothalamus of male subjects with obesity, expression of proUGN was lower when compared to subjects with normal body weight. Observed differences were not found in female brains. The gender differences existed in expression of the receptor as well. In male brain, expression of GC-C was lower in BA9 but higher in hypothalamus when compared to expressions in female brain. In BA10, BA11 and hypothalamus, GC-C expressions were in negative correlation to the volume of stomach content but only in male brain. This study suggests different role of brain UGN and GC-C in male and female individuals. At least in male brain UGN and GC-C are possibly involved in feeding regulation and obesity. In laboratory animals we can also see sex differences in proUGN and GC-C expression. Activation of brown adipose tissue is not only depending on the sex then also varies depending on the phase of estrous cycle. In this study we showed importance of studies in both male and female participants.

KEYWORDS: mouse and human brain, hypothalamus, prefrontal cortex, obesity, type 2 diabetes mellitus, glucose homeostasis

This study was co-financed by the European Union through the European Regional Development Fund, Operational Programme Competitiveness and Cohesion, grant agreement no. KK.01.1.1.01.0007, CoRENeuro and by the Croatian Science Foundation research grant (IP-2018-01-7416).

Bradykinin type 2 receptor deficiency alters vascular endothelial growth factor and atrial natriuretic peptide levels in early-stage diabetic retinopathy

Marin Radmilović[1], Helena Justić[2,3], Anja Barić[2,3], Martina Ratko[2,3], Iva Šimunić[2,3], Zoran Vatavuk[1], Aleksandra Dugandžić[4], Marina Dobrivojević Radmilović[2,3]

[1] Sestre milosrdnice University Hospital Center, Department of Ophthalmology, Vinogradska cesta 29, 10000, Zagreb, Croatia
[2] University of Zagreb School of Medicine, Croatian Institute for Brain Research, Šalata 12, 10000, Zagreb, Croatia
[3] University of Zagreb School of Medicine, Department of Histology and Embryology, Šalata 3, 10000, Zagreb, Croatia
[4] University of Zagreb School of Medicine, Department of Physiology, Šalata 3, 10000, Zagreb, Croatia

INTRODUCTION: Diabetic retinopathy (DR) is a leading cause of visual impairment among working-age adults. Anti-vascular endothelial growth factor (VEGF) therapy, the standard treatment for DR, has shown limited efficacy, raising concerns about possible neurodegenerative effects. In an effort to identify alternative therapeutic options, we explored the effects of bradykinin type 2 receptor (B2R) loss on the signaling pathways of VEGF and atrial natriuretic peptide (ANP), and on the development of retinal vascular permeability in a type 1 diabetes mouse model of early DR.

METHODS: Glycemic status assessment, intraocular (IOP) and blood pressure measurements, and fluorescein angiography were performed in male diabetic (Akita) mice, diabetic B2R knockout (Akita/B2R-KO) mice, and their non-diabetic controls (WT and B2R-KO; n = 18 per group) at 2 and 6 months of age. At 6 months, vascular permeability tests and magnetic resonance imaging were performed, and samples were collected for immunohistochemistry, ELISA, and qPCR analyses.

RESULTS: Diabetic B2R-deficient mice showed an exacerbated diabetic phenotype, but reduced IOP compared to diabetic Akita mice ($p<0.01$). Surprisingly, B2R deficiency led to increased vascular permeability ($p<0.01$), although no obvious fluorescein leakage was observed in angiograms. On the other hand, B2R deficiency attenuated ganglion cell loss ($p<0.05$) and retinal thinning ($p<0.01$). Furthermore, we showed that B2R deficiency decreases VEGF and VEGFR-2 protein levels ($p<0.001$), and combined with diabetes, significantly boosts ANP ($p<0.001$) and GC-A receptor ($p<0.0001$) expression, which could account for the improved neuroprotection.

CONCLUSION: This study highlights a novel regulatory role of B2R in VEGF and ANP signaling, suggesting potential targets for future research on vascular permeability and neuroprotection in early-stage DR.

ACKNOWLEDGMENTS: This work was funded by the Croatian Science Foundation project BRADISCHEMIA (UIP-2017-05-8082). Magnetic resonance imaging was done at the Laboratory for Regenerative Neuroscience - GlowLab, University of Zagreb School of Medicine.

The role of gut microbiota in circadian rhythm of bile acid levels

Darja Flegar[1,2]

[1] *Alimentary Pharmabiotic Centre, University College Cork, Cork, Ireland*
[2] *Department of Microbiology, University College Cork, Cork, Ireland*

Granulysin and perforin mediated cytotoxicity in patients with colorectal cancer

Ines Mrakovčić-Šutić[1,2]

[1] Department of Physiology, Immunology and Pathophysiology, University of Rijeka, Medical Faculty, Rijeka, Croatia
[2] Department of Basic Medical Sciences, University of Rijeka, Faculty of Health Studies, Rijeka, Croatia

Decoding chronic graft-versus-host disease with monocyte signatures and plasma chemokines

Antonija Babić[1], Ana Zelić Kerep[2], Antonela Lelas[2], Josip Knežević[1], Ivan Sabol[3], Lana Desnica[2], Nadira Duraković[2,4], Radovan Vrhovac[2,4], Dražen Pulanić[2,4], Drago Batinić[5], Hrvoje Lalić[1,5,6]

[1] Department of Laboratory Immunology, Clinical Department of Laboratory Diagnostics, University Hospital Center Zagreb, Zagreb, Croatia
[2] Division of Hematology, Department of Internal Medicine, University Hospital Centre Zagreb, Zagreb, Croatia
[3] Division of Molecular Medicine, Rudjer Boskovic Institute, Zagreb, Croatia
[4] Department of Internal Medicine, University of Zagreb School of Medicine, Zagreb, Croatia
[5] Department of Physiology, University of Zagreb School of Medicine, Zagreb, Croatia
[6] Laboratory for Cell Biology, Croatian Institute for Brain Research, University of Zagreb School of Medicine, Zagreb, Croatia

INTRODUCTION: Chronic graft-versus-host disease (cGVHD) complicates allogeneic hematopoietic stem cell transplantation (allo-HSCT). Monocytes contribute to its biology, but reports disagree on subset shifts; chemokine axes such as CX3CR1/fractalkine have been proposed to mediate trafficking. We tested whether circulating monocyte features and monocyte-related cytokines reflect cGVHD and its clinical phenotype.

AIM: To associate cGVHD status, severity and organ involvement with monocyte subsets/activation and plasma cytokines.

METHODS: Allo-HSCT recipients with (n=69) and without (n=32) cGVHD at the University Hospital Centre Zagreb, Croatia (2017–2023) were profiled. Flow cytometry enumerated classical (CD14++CD16–), intermediate (CD14++CD16+), and non-classical (CD14+CD16++) monocytes; CCR2/CX3CR1; CD14++HLA-DR– monocytes; and HLA-DR density (MFI). A flow cytometer-based multiplex bead array quantified IL-4, IL-2, CXCL10, IL-1β, TNF-α, MCP-1, IL-17A, IL-6, IL-10, IFN-γ, IL-12p70, and IL-8. Clinical variables included NIH global score, Karnofsky index, and organ involvement.

RESULTS: Relative proportions of monocyte subsets were broadly similar between groups. By contrast, cGVHD featured higher absolute numbers of classical monocytes and an expanded CD14++HLA-DR– fraction, with reduced HLA-DR MFI on classical and intermediate monocytes; CCR2 and CX3CR1 were comparable. Plasma CXCL10 and MCP-1 were increased in cGVHD. IL-6 and IL-8 tracked with disease burden—positively with NIH global score and inversely with Karnofsky performance. Organ-linked patterns included lower IL-2 with genital tract involvement; lower IFN-γ and IL-12p70 with lung involvement; higher IL-17A with oral involvement; and CXCL10 lower with joint/fascia yet higher with liver involvement. Unexpectedly, IL-10, IL-6, IL-8, CXCL10, and MCP-1 showed positive associations with overall survival.

CONCLUSION: cGVHD is characterized by a monocytic deactivation signature (increased CD14++HLA-DR– and reduced HLA-DR density) and a chemokine-rich milieu (CXCL10, MCP-1). Together with IL-6/IL-8 severity links and organ-specific cytokine patterns, these data suggest pragmatic biomarkers for monitoring and risk stratification after allo-HSCT and warrant prospective validation.

Acute hyperglycemia and (micro)vascular function: impact of experimental model, vessel size, sex and methodological approach

Helena Lenasi and Lana Kralj

Institute of Physiology, Faculty of Medicine, University of Ljubljana, Slovenia,

e-mail: helena.lenasi.ml@mf.uni-lj.si

It is well established that chronic hyperglycemia impairs endothelial function and vasomotion, likely through increased oxidative stress, inflammation, and altered autonomic nervous system activity. Also acutely elevated plasma glucose concentration has been shown to induce endothelial dysfunction in patients with diabetes or glucose intolerance, involving many interrelated mechanisms, including mitochondrial polarization, increased protein kinase C activity, and increased reactive oxygen species production, leading to endothelial nitric oxide (NO) synthase uncoupling and reduced NO bioavailability. However, the acute hyperglycamia-induced effects on vascular and microvascular function, particularly the NO-independent endothelial vasodilation, remain controversial, especially when considering different methodological approaches. Moreover, the precise time frame of plasma glucose increase after glucose loading is not clear, nor is it clear whether and how concomitant intake of other nutrients impacts (micro)vascular function. Besides from glucose per se, also insulin secreted in response to increased plasma glucose concentration importantly affects vascular function, further hampering data interpretation. It is thus important to consider all these effects when designing studies involving acute hyperglycemia and interpreting the results. Accordingly, the talk would expose some relevant thoughts and problems and present important differences in methodological approaches, when assessing the reactivity of isolated vessels versus in vivo assessment, in animal models and humans, respectively, and discuss potential effects of different time-frames and sex differences.

SHORT TALKS:

Association between pulse wave velocity and cardiovascular risk factors in patients with spondyloarthritis

Vedrana Tudor Špalj[1,2], Ana-Marija Laškarin[3], Vedrana Drvar[4], Tatjana Kehler[5,6] Iva Uravić Bursać[5], Silvija Miletić Gršković[5], Viktor Peršić[5,7], Gordana Laškarin[5,8]

[1] Department of Family Medicine, Faculty of Medicine, University of Rijeka, Rijeka, Croatia.
[2] Health Center of the Primorje-Gorski Kotar County, Rijeka, Croatia.
[3] PhD student, School of Dental Medicine, University of Zagreb, Zagreb, Croatia.
[4] Clinical Institute of Laboratory Diagnostics, University Hospital Centre. Rijeka, Croatia.
[5] Special Hospital for the Rehabilitation of Heart, Lung, and Rheumatic Diseases. Thalassotherapia-Opatija, Opatija, Croatia.
[6] Faculty of Health Studies, University of Rijeka, Rijeka, Croatia.
[7] Department of Rehabilitation and Sports Medicine, Faculty of Medicine, University of Rijeka, Rijeka, Croatia.
[8] Department of Physiology, Immunology and Pathophysiology, Faculty of Medicine, University of Rijeka, Rijeka, Croatia.

INTRODUCTION: Spondyloarthritis (SpA) is a group of rheumatic diseases, which begin before the age of 45, and represents a significant risk for cardiovascular (CV) diseases. The Systematic Coronary Risk Evaluation 2, recommended for assessing the CV risk is not adapted for people younger than 40 years. Aortic stiffness predicts CV morbidity and mortality regardless of age.

AIM: to determine CV risk in patients with SpA by measuring aortic stiffness and to investigate the interrelationship of aortic stiffness with disease activity parameters and classic CV risk factors.

METHODS: In patients with SpA (n71), we determined aortic stiffness by measuring pulse wave velocity (PWv) with an oscillometer (Agedio® B900, IEM, Stolberg, Germany). We assessed clinical disease activity [number of tender and swollen joints of 68 and 66, respectively, morning stiffness, pain and psoriasis severity, Disease Activity Index for Psoriatic Arthritis (DAPSA) and Ankylosing Spondylitis Disease Activity Score (ASDAS)] and analyzed serum interleukin 17A (IL-17A) concentration with Enzyme-Linked Immunosorbent Assay. We recorded classic CV risk factors [age, body mass index, arterial blood pressure (BP), fasting blood glucose and lipid profile analyzed routinely (Cobas Pro analyzer, Roche Diagnostics, and Boehringer Mannheim)].

RESULTS: PWv did not correlate with any of the above SpA activity parameters, but it was positively correlated with systolic BP ($r=0.421$; $p<0.001$), pulse pressure ($r=0.441$; $p=0.001$), glycemia ($r=0.319$; $p=0.007$) and age ($r=0.872$; $p<0.001$). In multiple linear regression, significant predictors of PWv were age, systolic BP and pulse pressure, but not glycemia ($R2=0.775$; $p<0.001$).

CONCLUSION: Oscillometric PWV reflected age, arterial pressure and glycemia, and not parameters of SpA inflammatory activity, therefore PWv represents a good marker for the presence of classic CV risk factors in patients with SpA, which can be easily and quickly assessed by a general practitioner.

FUNDING: University of Rijeka Project PU-294 uniri-iz-25-192. Funded by the European Union – NextGeneration EU.

KEYWORDS: aorta, arterial stiffness, blood pressure, pulse wave velocity, spondyloarthritis

CONFLICT OF INTEREST STATEMENT: The authors declare that there are no conflicts of interest.

Metabolic impact of stearoyl-coenzyme A desaturase inhibition on acute myeloid leukemia cells

Klara Bardač[1], Alojzija Brčić[2], Sophie James[3], Hrvoje Lalić[2,4], Romana Penker[5], Giovanny Rodriguez Blanco[5], Dora Visnjic[2], Paolo Gallipoli[3], Vilma Dembitz[2,3]

[1] University of Zagreb School of Medicine, Zagreb, Croatia
[2] Laboratory for Cell Biology, Department of Physiology, Croatian Institute for Brain Research, University of Zagreb School of Medicine, Zagreb, Croatia
[3] Centre for Haemato-Oncology, Barts Cancer Institute, Queen Mary University of London, London, UK
[4] Department of Laboratory Immunology, Clinical Department of Laboratory Diagnostics, University Hospital Center Zagreb, Zagreb, Croatia
[5] Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Graz, Graz, Austria

Inhibition of stearoyl-CoA desaturase (SCD) disrupts the balance of saturated and unsaturated fatty acids and leads to the death of tumor cells. In solid tumors, this toxicity is associated with endoplasmic reticulum stress and lipid peroxidation, which activate apoptosis and ferroptosis. However, in acute myeloid leukemia (AML), these mechanisms account for only part of the observed effect. The aim of this study was to investigate the metabolic basis of AML cell sensitivity to pharmacological SCD inhibition. A metabolomic analysis was performed on AML cell lines following incubation with or without the SCD inhibitor SSI-4. In accordance with their highest sensitivity to SSI-4, MOLM-13 cells exhibited significant changes in metabolite concentrations, most notably in amino acids. A 13C-glucose tracing experiment demonstrated that the increased amino acid concentrations were not a result of enhanced synthesis. Additionally, Krebs cycle metabolites in the presence of SSI-4 utilized glucose less as a carbon source, indicating an increased reliance on amino acids in sensitive cells. Analysis of metabolite consumption from the culture media confirmed an increased uptake of amino acids. Finally, culturing SSI-4–treated MOLM-13 cells in media with varying availability of specific amino acids showed a reduction in apoptosis under limited availability—particularly of leucine, isoleucine, and glutamine—suggesting that the toxicity of SSI-4 arises from metabolic changes associated with increased amino acid uptake and is dependent on their extracellular availability.

Central control of sympathetic nerve activity in a rat: the role of spinal cord and forebrain centers

Željka Minić[1]

[1] *University of Rijeka, Faculty of Biotechnology and Drug Development, Rijeka, Croatia*

INTRODUCTION: Central control of sympathetic nerve activity (SNA) has been studied extensively in the past. The central control of regional SNA by the brainstem and lower brain centers has been studied, however, the influence of forebrain or spinal centers, on SNA is less well understood. Spinal cord is an important site for control of SNA but sympathetic recordings obtained in spinal animals have not been frequently studied.

AIM: The purpose of this study was to test the effect of forebrain and spinal centers on splanchnic SNA in male Spraque-Dawley rats. All the experiments presented in this study were performed at Wayne State University School of Medicine. Accordingly, all protocols and surgical procedures were reviewed and approved by the Wayne State University Institutional Animal Care and Use Committee.

METHODS: SNA was recorded in urethane anesthetized, pancuronium paralyzed, and ventilated rats before and after performing precollicular decerebration. Time and frequency domain of SNA was analyzed before and after decerebration in (i) intact, (ii) baroreceptor denervated, and (iii) chronically spinalized rats. For time domain parameters: overall integrative SNA, burst amplitude, and burst frequency were quantified before and after decerebration while for frequency domain parameters: power spectral density and coherence analyses were used to quantify frequency and correlation as a function of frequency, in sympathetic and hemodynamic recordings.

RESULTS: In both intact and baroreceptor denervated animals, precollicular decerebration resulted in an immediate and dramatic increase in SNA of 126%±34% and 93%±38%, respectively. Spinal cord injury markedly altered the phenotype of SNA at baseline and abolished decerebration induced increases in SNA. The effect of precollicular decerebration on SNA firing frequency and coherence between SNA and hemodynamic parameters is presented.

CONCLUSION: The present data suggest that forebrain structures provide tonic descending inhibition of regional SNA and that this inhibition exists independently of central baroreflex processing.

POSTER ABSTRACTS

Does calcium salt of linseed oil affect concentration of essential fatty acids in goat milk?

Jasna Aladrović[1], Lana Pađen[1], Ivana Pejakić[2], Nataša Mikulec[2], Mladen Jarnjak[3], Krešimir Salajpal[2]

[1] Faculty of Veterinary Medicine, University of Zagreb, Heinzelova 55, Zagreb, Croatia
[2] Faculty of Agriculture, University of Zagreb, Svetošimunska 25, Zagreb, Croatia
[3] Fanon d.o.o., Petrijanec, Hrvatska

Lipid composition is one of the most important components of the nutritional quality of goat milk. The concentration of fat in milk depends on factors such as breed, nutrition, individual traits, and lactation period. Goat's milk production generally peaks six to nine weeks after kidding, while feed intake peaks around fifth month of milking. Therefore, does usually suffer from negative energy balance in early to mid-lactation. Body reserves are mobilised to make up for this energy deficit. Diet fat supplementation is most prevalent method to overcome energy deficit. To determine the effects of diets supplemented with protected fat on milk fatty acids (FAs) concentration 96 lactating primiparous Alpine does (60-90 day in lactation) were randomly divided into groups. Control group was fed the control diet (concentrate feed mixture with 18% of crude protein, grass and alfalfa hay), linseed oil (LO) was fed control diet + 43 g/daily of Ca salt of linseed oil (INSOMA LEIN, Fanon d.o.o., Croatia) and diet of palm oil (PO) group consisted of control diet + 33 g Ca salt of palm oil (Magnapac, Norel, Spain). Milk was collected as pooled samples for each group before supplementation, 7th, 14th and 21st day of supplementation. After extraction of total lipids, and transmethylation process, FAs composition was determined by GC. Milk collected in LO group consisted of higher concentration of FAs important in the development of specific milk flavour in goats (butyric, caproic, caprylic and capric acid) as well as linoleic acid (LA). Supplementation in LO group did not affect concentration of a-linolenic acid (ALA), arachidonic acid and eicosapentaenoic acid (EPA), whereas docosahexaenoic acid (DHA) and EPA+DHA were higher after 21 day of supplementation. The LA/ALA ratio also was higher in milk of LO group. We can conclude that supplementation with Ca salt of linseed oil elevate concentration of some essential FAs and improve nutritional value of goat milk.

Keywords: goat's milk, linseed oil, palm oil, fatty acids composition

Replication Stress–Induced Acute Myeloid Leukemia Differentiation is Governed by Nucleotide Metabolism and Ribonucleotide Reductase Composition

Alojzija Brčić[1], Hrvoje Lalić[1,2], Vilma Dembitz[1], Tomislav Smoljo[1], Romana Penker[3], Giovanny Rodriguez Blanco[3], Dora Višnjić[1]

[1] Laboratory for Cell Biology, Department of Physiology, Croatian Institute for Brain Research, University of Zagreb School of Medicine, Zagreb, Croatia
[2] Department of Laboratory Immunology, Clinical Department of Laboratory Diagnostics, University Hospital Center Zagreb, Zagreb, Croatia
[3] Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Graz, Graz, Austria

INTRODUCTION**:** Differentiation therapy represents a promising approach in acute myeloid leukemia (AML), particularly in older patients where cytotoxic treatments are poorly tolerated. DNA replication stress (RS) can drive leukemic cells toward differentiation rather than apoptosis. We previously observed that 5-aminoimidazole-4-carboxamide ribonucleoside (AICAr) promotes AML differentiation, but the mechanisms underlying this effect remain unclear.

AIM: This study aimed to systematically compare AICAr with DHODH inhibition (brequinar) and low-dose cytarabine (AraC) to define shared and distinct metabolic mechanisms underlying RS-induced differentiation.

METHODS: AML cell lines U937 (p53-mutant) and MOLM-13 (p53 wild-type) were treated with AICAr, brequinar, or AraC. Metabolomic profiling was performed using HILIC-LC-MS/MS, and cell cycle progression and differentiation markers (CD11b/CD64) were assessed by flow cytometry. Ribonucleotide reductase (RNR) subunit expression was modulated via siRNA or pharmacologic inhibitors (COH29, hydroxyurea) to determine their functional contributions to differentiation.

RESULTS: Low-dose AICAr induced biphasic UMP synthase inhibition and purine perturbations, promoting differentiation, whereas brequinar selectively inhibited DHODH and AraC increased dNTP pools. All agents caused S-phase arrest and disrupted nucleotide balance; these effects were abolished by high-dose ribo-/deoxyribonucleosides. RRM2 expression increased in U937 cells, while MOLM-13 predominantly induced p53-responsive RRM2B. RRM2 knockdown enhanced differentiation in U937 but impaired differentiation and reduced viability in MOLM-13, whereas RRM2B depletion had minimal effect. Pharmacologic RNR inhibition mirrored these divergent outcomes.

CONCLUSIONS: AML differentiation under replication stress is tightly linked to nucleotide metabolism, with RNR subunit composition and checkpoint competency determining outcomes. While AICAr, brequinar, and AraC consistently induce differentiation, the balance between RRM2 and RRM2B dictates whether replication stress drives productive maturation or replication collapse. These findings highlight nucleotide homeostasis and RNR regulation as central determinants of AML differentiation and suggest that combining metabolic targeting with checkpoint-modulating strategies could enhance therapeutic efficacy.

Frontal theta synchrony, parietal P300, and entropy reveal nonlinear network dynamics under increasing cognitive load in a visual oddball task

Filip Ileš[1], Tena Popović[1], Nikola Habek[1]

[1] *Croatian Institute for Brain Research, University of Zagreb School of Medicine, Zagreb, Croatia*

Cognitive workload is commonly assessed through subjective ratings, yet neural measures may uncover hidden states of instability not captured by self-reports. We combined EEG and NASA-TLX to examine how increasing task complexity modulates frontal–parietal dynamics during a visual oddball paradigm.

Five healthy participants performed three blocks of increasing difficulty (target detection among 6, 9, and 12 letters). EEG data were preprocessed by filtering and Independent Component Analysis (ICA) with artifact and bad epoch rejection. Frontal theta inter-trial coherence (ITCθ) and parietal P300 amplitudes (Target–Standard) were extracted as markers of salience detection and conscious evaluation, respectively, while spectral entropy was computed as an index of neural complexity. Subjective workload was assessed after each block using NASA-TLX.

Repeated-measures ANOVA revealed significant block effects on both EEG markers. Frontal ITCθ ($F(2,8)=13.5$, $p=0.003$) and parietal P300 amplitude ($F(2,6)=12.9$, $p=0.007$) both decreased under medium load (9-letter block); however, frontal ITCθ recovered under high load (12-letter block), whereas parietal P300 amplitude remained low. In contrast, NASA-TLX ratings increased monotonically with task complexity, reflecting perceived effort. Importantly, subjective frustration correlated positively with parietal spectral entropy differences (ΔH_pa; $\rho=0.625$, $p=0.030$), linking perceived workload with increased neural complexity of target processing.

In conclusion, subjective and neural measures captured distinct aspects of cognitive load. While TLX indicated a linear increase in effort, EEG revealed a nonlinear breakdown and partial recovery of frontal–parietal synchrony, with frustration aligning to entropy-based neural complexity. This multimodal approach highlights the added value of EEG in detecting hidden states of cognitive strain.

Keywords: EEG, visual oddball, cognitive load, frontal theta/parietal P300, spectral entropy

Psoriatic Arthritis and Ankylosing Spondylitis are driven by functionally different monocyte phenotypes

Ivo Krešić[1], Pavao Planinić[1], Sara Aničić[3,4], Marta Radošević[3,4], Sara Priselac[4,5], Tomislav Balen[4,5], Katerina Z. Petrović[4], Danka Grčević[3,4], Tomislav Kelava[3,4], Nataša Kovačić[4,5], Marina I. Matijašević[2], Ivan Ćavar[1], Alan Šućur[3,4]

[1] Department of Physiology, School of Medicine, University of Mostar, 88000 Mostar, Bosnia and Herzegovina,
[2] "Sveti Duh" University Hospital, Zagreb, Croatia,
[3] Department of Physiology, School of Medicine University of Zagreb, 10000 Zagreb, Croatia
[4] Laboratory for Molecular Immunology, Croatian Institute for Brain Research, School of Medicine, University of Zagreb, 10000 Zagreb, Croatia
[5] Department of Anatomy, School of Medicine University of Zagreb, 10000 Zagreb, Croatia

INTRODUCTION: Monocytes play a crucial role in the pathogenesis of spondyloarthropathies (SpA), such as ankylosing spondylitis (AS) and psoriatic arthritis (PsA), by differentiating into key inflammatory cells. Among these, monocyte-derived dendritic cells (moDCs) are of particular interest due to their close interaction with T-cells via the IL-12-23/IL-17 axis. This study aims to analyze the expression of DC markers on blood monocytes, evaluate changes upon their differentiation into DCs, and test their subsequent functionality.

METHODS: PBMCs were isolated from patients with active PsA, AS, and healthy controls. Using flow cytometry, we analyzed a panel of markers for monocyte lineage (CD14, CD16), DC differentiation (CD1a, CD1c, CD141, CD206, CD209), antigen presentation (CD40, MHCII), and costimulation (CD80, CD86). Sorted classical monocytes were cultured with DC-inducing factors, IL-17, or both; a control group underwent spontaneous differentiation. Phagocytic ability was tested via pHrodo assay, and antigen-presenting capacity was assessed in a moDC: T-cell co-culture by measuring cytokine production.

RESULTS: Fresh PsA monocytes expressed higher levels of CD206, CD209 and CD1a. In vitro, spontaneously differentiated AS monocytes showed lower CD141 and higher CD40. AS-derived moDCs had heightened phagocytosis but produced less IL-12/23, resulting in lower T-cell IFN-γ and TNF-α production. Conversely, PsA-derived moDCs showed higher expression of costimulatory (CD86) and dendritic (CD1c) markers. Across all groups, IL-17 supplementation increased CD209 while decreasing CD141 and CD1a expression.

CONCLUSION: Our findings reveal distinct pathological pathways in SpA. In psoriatic arthritis, monocytes and moDCs show a phenotype skewed towards heightened antigen presentation. In contrast, cells in ankylosing spondylitis display a profile characterized by enhanced phagocytosis but a reduced capacity to stimulate T-cell responses, suggesting different primary mechanisms of immune dysfunction between these two diseases.

Longitudinal in vivo assessment of cerebral ischemic damage development in bradykinin receptor type 2 deficient mice

Helena Justić[1, 2], Anja Barić[1, 2], Martina Ratko[1], Iva Šimunić[1, 2], Marta Pongrac[1,2], Siniša Škokić[1], Marina Dobrivojević Radmilović[1, 2]

[1] University of Zagreb School of Medicine, Croatian Institute for Brain Research, Zagreb, Croatia
[2] University of Zagreb School of Medicine, Department of Histology and Embryology, Zagreb, Croatia
The corresponding author: Helena Justić, helena.justic@mef.hr

INTRODUCTION: Bradykinin, a vasoactive and pro-inflammatory peptide released during cerebral ischemia has been shown to increase vascular permeability and exacerbate neurological damage. However, emerging evidence suggests that bradykinin receptor type 2 (B2R) activation may confer neuroprotection, indicating a possible dual role in ischemic pathology. Given potential links between B2R, glucose metabolism and post-ischemic outcomes, we conducted a longitudinal in vivo assessment using a mouse model of middle cerebral artery occlusion (MCAO) to evaluate the function of B2R in ischemic progression and possible involvement in metabolic regulation.

METHODS: Four-month-old male C57Bl/6J and B2R-deficient (B2R-KO) mice underwent a 30-minute intraluminal Koizumi MCAO to induce cerebral ischemia. Neurological scoring, behavioural testing and MRI were conducted 7 days before and at 2, 9, and 35 days post-MCAO. Blood glucose levels were measured pre- and post-ischemia. Image analysis was performed using ImageJ.

RESULTS: MRI revealed no significant differences in acute ischemic lesion volume between groups. However, 35 days after MCAO, B2R-KO mice exhibited significant ipsilateral hemisphere atrophy with pronounced neuronal loss, astrogliosis and increased arterial volume compared to controls. B2R deficiency was associated with worsened acute neurological deficits and impaired long-term neurological function. Additionally, blood glucose levels were influenced both by the absence of B2R and by the ischemic injury during the chronic phase.

CONCLUSION: Longitudinal multimodal analysis revealed that B2R deficiency leads to impaired brain function, poorer recovery and brain tissue loss, implying a protective role of B2R during both acute and chronic phase of ischemia. Furthermore, observed alterations in glycemic status indicate a metabolic function of B2R and underscore the potential impact of ischemic injury on long-term glucose control.

Acknowledgements: The study was funded by the Croatian Science Foundation project BRADISCHEMIA (UIP-2017 05-8082). Magnetic resonance imaging was done at the Laboratory for Regenerative Neuroscience - GlowLab, University of Zagreb School of Medicine.

Salivary interleukin-17A has diagnostic accuracy for internal temporomandibular joint disorders in patients with spondyloarthritis

Ana-Marija Laškarin[1], Vedrana Drvar[2], Stjepan Špalj[3,4], Emina Babarović[5], Gordana Laškarin[6,7], Tatjana Kehler[6,8], Nikša Dulčić[9]

[1] PhD student, Postgraduate doctoral study of Dental Medicine, School of Dental Medicine, University of Zagreb, Zagreb, Croatia
[2] Clinical Department of Laboratory Diagnostics, Clinical Hospital Centre Rijeka, Rijeka, Croatia
[3] Department of Orthodontics, Faculty of Dental Medicine, University of Rijeka, Rijeka, Croatia
[4] Department of Dental Medicine, Faculty of Dental Medicine and Health, J. J. Strossmayer, University of Osijek, Osijek, Croatia
[5] Department of Pathology, Clinical Hospital Center, University of Rijeka, Croatia
[6] Special Hospital for Rehabilitation of Heart, Lung and Rheumatism Diseases Thalassotherapia-Opatija, Opatija, Croatia
[7] Department of Physiology, Immunology and Pathophysiology, Faculty of Medicine, University of Rijeka, Rijeka, Croatia
[8] Faculty of Health Studies, University of Rijeka, Rijeka, Croatia
[9] Department of Removable Prosthodontics, School of Dental Medicine, University of Zagreb, Zagreb, Croatia

INTRODUCTION: Internal derangements (ID) of temporomandibular joints (TMJ) (disc displacements and degenerative joint disease) constitute a subgroup of temporomandibular disorders (TMD). Inflammation is the most common cause of ID, which also represents clinical manifestation of active axial spondyloarthritis (SpA) mediated by oral-intestinal dysbiosis and interleukin-17A. THE AIM was to investigate diagnostic accuracy of salivary IL-17A for ID in patients with SpA.

METHODS: Patients were diagnosed as symptomatic ID (n 64), asymptomatic ID (n 50), and healthy TMJ (control, n 86) based on intracapsular arthralgia, regardless of joint sounds using Diagnostic criteria (DC)/TMD protocol. We recorded ASDAS (Ankylosing Spondylitis Disease Activity Score), DAPSA (Disease Activity Index for Psoriatic Arthritis), patient's assessment of SpA activity and pain, lower jaw mobility, parafunctions of TMJ, somatization and anxiety, and measured salivary IL-17A using ELISA.

RESULTS: Salivary IL-17A showed diagnostic accuracy for symptomatic ID (cut off 11 pg/mL) and asymptomatic ID (cut off 11.6 pg/mL) with high sensitivity (68.75% or 80%, respectively) and specificity (83.72% or 87.2%, respectively). Patients with salivary IL-17A above the 11 pg/mL expressed more frequently painful ID, local myalgia, myofascial pain and TMP-related headache, somatisation and anxiety, and showed more limited lower jaw movements than patients with IL-17A ≤ 11 pg/mL. They also self-reported greater spinal pain and SpA activity, as corresponded to DAPSA. The group with IL-17A > 11.6 pg/mL for diagnosis asymptomatic ID had also increased activity of SpA (ASDAS, spine pain), and expressed more frequently painless ID accompanied with joint sounds, headache attributed to TMD, which contributed to extra-articular pain over last 30 and 160 days.

CONCLUSION: Symptomatic and asymptomatic ID are associated with the activity of SpA, as pain and joint noises are predictors of TMD severity.

Funding: University of Rijeka Project PU-294 uniri-iz-25-192. *Funded by the European Union – NextGenerationEU.*

Keywords: interleukin-17A, internal derangements of temporomandibular joints, spondyloarthritis, temporomandibular disorders

Morphometric response of sow erythrocytes after in vitro exposure to 5G RF-EMR at 700 MHz

Nikolino Žura[1], Stefani Fruk[2], Marinko Vilić[2], Porin Perić[3,4], Silvijo Vince[5], Krešimir Malarić[6], Ana Shek Vugrovečki[2], Suzana Milinković Tur[2], Nina Poljičak Milas[7], Jadranka Pejaković Hlede[2], Ivona Žura Žaja[2]

[1] University of Applied Health Sciences, Zagreb, Croatia
[2] Unit of Physiology and Radiobiology, Faculty of Veterinary Medicine, University of Zagreb, Croatia
[3] University Hospital Center Zagreb, Department of Rheumatology and Rehabilitation, Zagreb, Croatia
[4] Faculty of Medicine, University of Zagreb, Croatia
[5] Clinic for Obstetric and Reproduction, Faculty of Veterinary Medicine, University of Zagreb, Croatia
[6] Department of Communication and Space Technologies, Faculty of Electrical Engineering and Computing University of Zagreb, Croatia
[7] Unit of Pathological Physiology, Faculty of Veterinary Medicine, University of Zagreb, Croatia

Abstract

The effects of radiofrequency electromagnetic radiation (RF-EMR) from 5G frequencies on erythrocyte (RBC) morphometric parameters in domestic animals are still poorly understood and insufficiently studied. The aim of this study was to evaluate the effects of 5G RF-EMR at 700 MHz on erythrocyte morphometry and to identify potential RBC subpopulations based on morphometric indicators after short-term in vitro exposure of sow blood.

Blood samples from 16 German Landrace sows (1–2 years) were collected from the vena cava cranialis. For each sow, one EDTA tube was designated as experimental and one as control. Experimental samples were exposed to continuous RF-EMR at 700 MHz, 4 h after sampling, in a Half-cone gigahertz transversal electromagnetic cell (10 V/m, 20–22 °C) for 2 h, while controls were kept under identical conditions without exposure. Smears were prepared immediately after treatment, stained with Pappenheim, and analyzed (>100 RBCs per smear) using the SFORM program. Morphometric indicators of erythrocyte size and shape were determined. The values for surface area, outline, minimum and maximum radius, convex area, length, width and contour index were significantly higher in the experimental group than in the control group and significantly lower for the solidity and form factor values.

Exposure at 700 MHz resulted in more elongated RBCs with irregular borders. Cluster analysis identified two subpopulations: ES1 (smaller, circular, compact cells) and ES2 (larger cells with irregular borders). The proportion of ES2 was slightly higher in the exposed group (17.0% vs. 15.9%), while the proportion of ES1 was lower (83.0% vs. 84.1%) compared to controls; however, these differences were not statistically significant ($p = 0.30$).

These results suggest that in vitro exposure of blood to 5G RF-EMR at 700 MHz may affect RBC shape and subpopulation distribution, with possible consequences for their mechanical properties, membrane integrity and function.

Key words: 5G radiofrequency electromagnetic radiation; in vitro exposure; sows' blood; erythrocyte morphometry; erythrocyte subpopulations