RECOOP HST ASSOCIATION

Cedars Sinai

21st Bridges in Life Sciences Conference
11th Science and Art Exhibition
March 25-26, 2026
Zagreb, Croatia

Sheraton Zagreb Hotel

21st RECOOP Bridges in Life Sciences

Conference

March 25 - 26, 2026

Zagreb, Croatia

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Seeds in the pods
Ines Drenjančević, J. J. Strossmayer University of Osijek, Osijek, Croatia

Agenda

21st RECOOP Bridges in Life Sciences Conference

March 25 - 26, 2026
Zagreb, Croatia

Sheraton Zagreb Hotel
Kneza Borne 2, Zagreb 10000, Croatia

March 24, 2026

18:00 – 18:30 Registration
19:00 – 22:00 Welcome Dinner
21:00 – 21:15 Briefing for Session Chairs

March 25-26, 2026

21st RECOOP Bridges in Life Sciences Conference – Sessions:

- Drug Development and Effects (DDE)
- Neurodegenerative Diseases (NDD)
- Metabolic Disorders (MTD)
- Cardiovascular Diseases (CVD)
- Cedars – RECOOP Research Centers (CRRCs)
- Medical Imaging (MI)
- Translational Medical Research (TMR)
- Reproductive Health (RPH)
- Infections and Immunology (IFI)
- Nanomedicine and Safety (NMS)
- Clinical and Epidemiological Research (CER)

Presentations
Scientists: 10 minutes oral ppt presentation.
Undergraduate and PhD students: 5 minutes oral ppt presentation.

Discussion will be at the end of each block.
Sessions are moderated by the session chairs.

March 27, 2026
Departure

March 25, 2026

21st RECOOP Bridges in Life Sciences Conference

8:30 – 9:00 Registration

9:00 – 9:15 Opening

Róbert Gáspár, RECOOP HST Association & Albert Szent-Györgyi Medical School, University of Szeged, Hungary
David M. Wrigley, Cedars-Sinai Medical Center, Los Angeles, CA, USA

9:15 – 10:50 Drug Development and Effects (DDE)

Session Chairs ***Mykhaylo Korda***
Eszter Ducza

Anticancer drugs: Design, development, and action
Rostyslav Stoika, Institute of Cell Biology NAS, Lviv, Ukraine

Molecular hydrogen modulates inflammatory and immune markers in the tumour microenvironment of experimental colorectal cancer
Mykhaylo Korda, I. Horbachevsky Ternopil National Medical University, Ternopil, Ukraine

Cell encapsulation for wound healing: Storage and transportation of mesenchymal stem cells at ambient temperature
Oleksandr Petrenko, Institute for Problems of Cryobiology and Cryomedicine NAS, Kharkiv, Ukraine

Modulation of calreticulin by the thiazolidinone derivative Les-6650 as a therapeutic strategy for myeloproliferative neoplasms
Nataliya Finiuk, Institute of Cell Biology NAS, Lviv, Ukraine

Antifibrotic activity of juglone-based thiopyrano[2,3-D]thiazole in TGF-β1-stimulated fibroblasts model
Iryna Ivasechko, Institute of Cell Biology NAS, Lviv, Ukraine

Nongenomic effects of 17β estradiol on gastric smooth muscle: Sex difference and signaling mechanism in rats
Mohammed Abdalla, University of Szeged, Szeged, Hungary

Smooth muscle electromyography for assessing gastroparesis induced by vagotomy, dexamethasone, and pantoprazole in rats
Ayman Mohammed, University of Szeged, Szeged, Hungary

Effects of dextromethorphan on neurotransmission in rat synaptosome
Zoltán Lincmajer, Semmelweis University, Budapest, Hungary

Cell death and DNA damage in human esophageal carcinoma cells exposed to low-energy protons
Natália Maťašeje, Comenius University, Bratislava, Slovakia

Discussion: 20 minutes

10:50 – 11:20 Coffee Break & 11th Science and Art Exhibition

11:20 – 12:20 Neurodegenerative Diseases (NDD)

Session Chairs ***Oleksandr Petrenko***
Marija Heffer

Psychological and cognitive consequences of war experience in combat soldiers and veterans
Olha Liuta, I. Horbachevsky Ternopil National Medical University, Ternopil, Ukraine

Comparative characteristics of psychological mechanisms of resilience and stress tolerance in combatants and veterans
Olena Venger, I. Horbachevsky Ternopil National Medical University, Ternopil, Ukraine

Toxic effect of Cu excess in brain nerve terminals and its neutralization
Tatiana Borisova, Palladin Institute of Biochemistry NAS, Kyiv, Ukraine

Impact of military life and gender on glycan age
Petar Šušnjara, J. J. Strossmayer University of Osijek, Osijek, Croatia

Ischemia-induced proteomic changes in the mouse cortex: Discovery of new markers for neural damage and repair
Monika Berecki, University of Zagreb School of Medicine, Zagreb, Croatia

Heterotypic neuro-mesenchymal spheroids as a promising 3D model of neuroregeneration
Olga Maiorova, Institute for Problems of Cryobiology and Cryomedicine NAS, Kharkiv, Ukraine

Discussion: 10 minutes

12:20 – 13:00 Metabolic Disorders (MTD)

Session Chairs ***Éva Szökő***
Senka Blažetić

Mapping the diabetic gestation: Fetal cardiac ultrasound and maternal–fetal characteristics
Andrea Surányi, University of Szeged, Szeged, Hungary

Modulatory effect of molecular hydrogen on oxidative stress, glycemic and lipid profiles in metabolic syndrome
Oksana Shevchuk, I. Horbachevsky Ternopil National Medical University, Ternopil, Ukraine

Inhibition of ganglioside degradation affects insulin signalization and SH-SY5Y cell morphology
Milorad Zjalić, University of Zagreb School of Medicine, Zagreb, Croatia

Discussion: 10 minutes

13:00 - 14:00 Lunch Break

14:00 – 15:00 Cardiovascular Diseases (CVD)

Session Chairs ***Volodymyr Chernyshenko***
Ines Drenjančević

The prognostic value of arginase II determination in patients with arterial hypertension
Lesya Kobylinska, Danylo Halytsky Lviv National Medical University, Lviv, Ukraine

Thrombosis predisposition in PTSD patients
Volodymyr Chernyshenko, Palladin Institute of Biochemistry NAS, Kyiv, Ukraine

Quantitative detection of early soluble fibrin in blood plasma using I-5A antibody
Roksolana Demchynska, Palladin Institute of Biochemistry NAS, Kyiv, Ukraine

Altered expressions of miRNAs involved in the regulation of antioxidant enzymes' expression in patients with hypertension and chronic kidney disease
Ana Marinčić Žagar, J. J. Strossmayer University of Osijek, Osijek, Croatia

Obtaining and characterization of proteinase with fibrin(ogen)olytic activity from the culture medium of Bacillus sp. L9
Yelyzaveta Huslieva, Palladin Institute of Biochemistry NAS, Kyiv, Ukraine

Properties of a new haemostatic agent based on modified collagen matrices
Artem Starodubets, Palladin Institute of Biochemistry NAS, Kyiv, Ukraine

The splenectomy influences the outcomes of ischemic brain lesion in the mouse
Dominik Romić, University of Zagreb School of Medicine, Zagreb, Croatia

Discussion: 15 minutes

15:00 – 16:00 Cedars – RECOOP Research Centers (CRRCs)

Session Chairs ***Róbert Gáspár***
Oksana Shevchuk
Sandor G. Vari

Comparison of acute and chronic post-traumatic stress disorder in military, Lviv, Ukraine
Sandor G. Vari, Cedars-Sinai Medical Center, Los Angeles, USA
Nataliia Drobinska, Danylo Halytsky Lviv National Medical University, Lviv, Ukraine

Discussion: 5 minutes

Ozonated water and oils as adjunct antimicrobials: sublethal damage, biofilm reduction, and antibiotic synergy
Taras Pyatkovskyy, I. Horbachevsky Ternopil National Medical University, Ternopil, Ukraine

Discussion: 5 minutes

The role of glycosylation in neurodegenerative disease biomarker research, focusing on low-grade inflammation
András Guttman, University of Debrecen, Debrecen, Hungary

Discussion: 5 minutes

Reprogramming the endothelium: Vasoprotective and anti-senescent effects of alpha-linolenic acid
Nikolina Kolobarić, J. J. Strossmayer University of Osijek, Osijek, Croatia

Discussion: 5 minutes

16:30 – 17:30 21st General Assembly Meeting

RECOOP HST Association – invited participants only

March 26, 2026

21st RECOOP Bridges in Life Sciences Conference

08:40 – 09:00 Review of the 21st General Assembly Meeting

Róbert Gáspár, President of the RECOOP HST Association
Laudation

09:00 – 10:15 Medical Imaging (MI)

Session Chairs ***Srećko Gajović***
Bartosz Krajnik

All-in-one polymer-engineered lanthanide-based upconverting nanoparticles for bioimaging
Daniel Horák, Institute of Macromolecular Chemistry CAS, Prague, Czech Republic

Long-term *in vivo* evaluation of ischemic brain damage evolution in bradykinin B2 receptor–deficient mice
Helena Justić, University of Zagreb School of Medicine, Zagreb, Croatia

Real-time label-free phase imaging with digital holographic microscopy
Bartosz Krajnik, Wroclaw University of Science and Technology, Wroclaw, Poland

Water-dispersible single-particle upconverting nanothermometers stabilized with imidazolium-based ionic liquids
Magdalena Święs, Wroclaw University of Science and Technology, Wroclaw, Poland

Molecular docking dynamics in nanopores using Defocused Wide-field Imaging
Nikola Rybarczyk, Wroclaw University of Science and Technology, Wroclaw, Poland

Advancing biological image quantification through machine learning driven segmentation
Iva Šimunić, University of Zagreb School of Medicine, Zagreb, Croatia

Early sonographic triad leading to first-trimester diagnosis of Meckel–Gruber syndrome – case report
Adina-Elena Nenciu, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania

Fetal speckle tracking echocardiography in pregnancies with intrauterine growth restriction
Adrian-Valeriu Neacşu, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania

Discussion: 15 minutes

10:15 – 10:50 Translational Medical Research (TMR)

Session Chairs ***Tamás Tábi***
Oksana Shevchuk

Bradykinin type 2 receptor deficiency reshapes acute neuroinflammation and improves cell survival after ischemic stroke in diabetic mice
Dinko Smilović, University of Zagreb School of Medicine, Zagreb, Croatia

Sex differences in oxidative stress and myocardial morphology in a rat model of post-traumatic stress disorder
Viktoria Miroshnyk, I. Horbachevsky Ternopil National Medical University, Ternopil, Ukraine

Validation of a new automated method for determining overall hemostatic potential
Anastasiia Pavlenko, Palladin Institute of Biochemistry NAS, Kyiv, Ukraine

The effect of frozen and lyophilized human cord blood serum on L929 cells
Valeriia Hoidina, Institute for Problems of Cryobiology and Cryomedicine NAS, Kharkiv, Ukraine

Discussion: 10 minutes

10:50– 11:15 Coffee Break & 11th Science and Art Exhibition

11:15 – 12:30 Reproductive Health (RPH)

Session Chairs ***Tibor Ertl***
Iuliana Ceausu

Assessing corticosteroid effects on fetal lung development using shear wave elastography in preterm birth risk
Réka A. Vass, University of Pecs, Pecs, Hungary

Investigating the functions of aquaporins in regulating uterine contractions and preventing preterm birth
Kata Kira Kemény, University of Szeged, Szeged, Hungary

The non-genomic actions of 5α- and 5β-dihydrotestosterone on a rat uterus model: in vitro and in vivo study
Saif-alnasr H. Mohammed, University of Szeged, Szeged, Hungary

Impact of pasteurization on miRNA composition in human breast milk
Joshua Klemt, University of Pecs, Pecs, Hungary

The effect of artificial reproductive techniques on the hormonal status of Newborns
Dora Filarszky, University of Pecs, Pecs, Hungary

Impact of metabolic and hypertensive pregnancy conditions on placental ABC transporters
Péter Szatmári, University of Szeged, Szeged, Hungary

Pregnancy after breast cancer management in a young woman: Case report
Maria Videnie, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania

The benefits and outcome of prenatal whole exome testing (WES) in a Romanian cohort
Ileana-Delia Manea-Săbău, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania

Impact of prenatal stress and cigarette smoke exposure on early neurobehavioral development in rats: A combined DOHAD perspective
Barbara Mammel, University of Pecs, Pecs, Hungary

Placental alpha microglobulin-1 (PartoSure) test for the prediction of preterm birth
Rachamim Karo Yakov, University of Szeged, Szeged, Hungary

Discussion: 15 minutes

12:30 – 13:00 Infections and Immunology (IFI)

Session Chairs ***Jana Tulinská***
András Guttman

Pediatric COVID-19 and MIS-C: Unseen endocrine shifts
Kateryna Kozak, I. Horbachevsky Ternopil National Medical University, Ternopil, Ukraine

Investigation of EV-DNA in juvenile idiopathic arthritis
Kristína Lichá, Comenius University, Bratislava, Slovakia

Seasonal dynamics of childhood Community-acquired pneumonia admitted at Lviv Infection Diseases Clinic: Marked surge and shifting patterns in 2024–2025
Melaniia Iryna Nadraga, Danylo Halytsky Lviv National Medical University, Lviv, Ukraine

Discussion: 5 minutes

13:00 – 14:10 Lunch Break

14:10 – 15:00 Nanomedicine and Safety (NMS)

Session Chairs ***Daniel Horák***
Rostyslav Stoika

Sub-chronic copper oxide nanoparticle inhalation alters adaptive and innate immunity in mice
Jana Tulinská, Slovak Medical University, Bratislava, Slovakia

Theranostic upconversion nanoparticles for cancer treatment
Oleksandr Shapoval, Institute of Macromolecular Chemistry CAS, Prague, Czech Republic

Polymer-modified upconverting nanoparticles for multimodal imaging and hypericin delivery
Taras Vasylyshyn, Institute of Macromolecular Chemistry CAS, Prague, Czech Republic

Multicore nanoassemblies for enhanced magnetic hyperthermia
Anna Hlukhaniuk, Institute of Macromolecular Chemistry CAS, Prague, Czech Republic

Neuro-safe biochar from agricultural waste
Liliia Kalynovska, Palladin Institute of Biochemistry NAS, Kyiv, Ukraine

Discussion: 15 minutes

15:00 – 15:30 Coffee Break & 11th Science and Art Exhibition

15:30 – 16:15 Clinical and Epidemiological Research (CER)

Session Chairs ***Radana Gurecká***
Norbert Buzás

Technology acceptance as a psychosocial determinant in the telerehabilitation of patients with metabolic syndrome
Norbert Buzás, University of Szeged, Szeged, Hungary

Preferred features of continuous glucose monitoring sensors: A conjoint analysis-based user preference study
Tamás Ujházi, University of Szeged, Szeged, Hungary

Relationship between nutritional status and psychological distress in medical students in Slovakia
Radana Gurecká, Comenius University, Bratislava, Slovakia

Spectrum and frequency of wheezing causes in toddlers
Denys Lialiuk, Danylo Halytsky Lviv National Medical University, Lviv, Ukraine

Discussion: 10 minutes

16:15 – 16:30 Closing Remarks

Róbert Gáspár, President of the RECOOP HST Association
David M. Wrigley, Cedars-Sinai Medical Center, Los Angeles, USA

Conference photo

19:00 Farewell Dinner

Thank you!

21st RECOOP Bridges in Life Sciences

Conference

Abstracts by Sessions

March 25 - 26, 2026

Drug Development and Effects (DDE)

Goldfish in Aerosol (pic from optical particle counting)

Renáta Lehotská
Slovak Medical University, Bratislava, Slovakia

Anticancer Drugs: Design, Development, and Action

Stoika R.

Department of Regulation of Cell Proliferation and Apoptosis, Institute of Cell Biology, NAS of Ukraine, Lviv, Ukraine

Corresponding author: Rostyslav Stoika, stoika.rostyslav@gmail.com

Keywords: anticancer drugs, design, development, biological action

Aims: The design and development of anticancer drugs was addressed with focusing on heterocyclic compounds whose metrics clearly demonstrate their dominance among these drugs. Five-member heterocyclic rings are used as structural blocks in synthesis of most drug-like compounds.
Methods: In order to enhance the activity, improve water-solubility, transportation and biocompatibility of heterocyclic compounds with anticancer activity, two main approaches are applied: 1) covalent modification via: a) synthesis of hybrid molecules with other compounds; b) changes in side chemical groups of heterocycles; 2) non-covalent modification via: a) immobilization of the compound on micellar amphiphilic polymers; b) incorporation into liposomes; c) immobilization in carbon nanotubes or Fullerene C60 nanoparticles; d) absorption by mineral (Au, Iron Oxides, zeolite) nanoparticles; e) others.
Results: In design and synthesis of new benzoisothiazole-1,2,3-triazole-4-carboxamide conjugates, pharmacophore-based hybridization was implemented to combine biologically active scaffolds via piperazine linker (1). Steric factor of the substituent (CH_3) in position 5 on the toxicity indicator (IC50) of created triazolic compounds toward human carcinoma MCF-7 cells was shown. IC50 of hybrid compounds 5h and 5j was comparable with that of the doxorubicin. However, the Selectivity Index (SI) of these compounds was 131.58 and 111.11, correspondingly, while doxorubicin did not show any selectivity (SI = 0.82 – 1.96). The capability of DNA intercalation and induction of DNA fragmentation by these compounds was comparable with that of the doxorubicin. Their DNA targeting action was confirmed in molecular docking *in silico* experiments using B–DNA dodecamer d(CGCGAATTCGCG)2 as a target for intercalation. In another series of experiments, conjugate of 4-thiazolidinone with natural compound Juglone was synthesized (Les-6400) (2) and its bio-isosteric derivatives Les-6547 i Les-6557 (3) were also designed and synthesized. These heterocyclic compounds are hybrid derivatives of thiopyrano[2,3-d]thiazoles with 5-hydroxy-1,4-naphthoquinone. Their anticancer potential and good bio-tolerance were demonstrated. They dose- and time-dependently decreased DNA biosynthesis, induced apoptosis (initiator (8, 9, 10) and effector (3/7) caspases, decreased membrane potential of mitochondria, and increased intracellular content of ROS in human colorectal tumor cells of different lines.
Conclusions: Covalent and non-covalent modifications of heterocyclic compounds were shown to improve their physic-chemical characteristics, enhance anticancer potential, and provide bio-tolerance.

Publications: 1. N. Pokhodylo, et al. J. Mol. Structure, 2024; 2. I. Ivasechko et al. Eur J Med Chem. 2023; 2. Ju. Kozak, et al. Cells 2025.
Studies were conducted according to scientific collaboration agreements with the teams of Prof. N. Pokhodylo (Lviv National University) and Prof. R. Lesyk (Lviv National Medical University).
Ethical Committee Approval: Not applicable.
Acknowledgements. The author thanks Cedars-Sinai Medical Center's International Research and Innovation in Medicine Program, and the Regional Cooperation for Health, Science and Technology Association (RECOOP HST Association) for their support.

Molecular Hydrogen Modulates Inflammatory and Immune Markers in the Tumour Microenvironment of Experimental Colorectal Cancer

Kramar S.B., Pokotylo O.O., Kravchuk Y.S., Shevchuk O.O., Korda M.M.*

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Keywords. colorectal cancer, molecular hydrogen, immunohistochemistry, tumour microenvironment

Introduction. Colorectal cancer (CRC) remains one of the most serious oncological problems worldwide. Despite continuous improvements, a significant proportion of patients still face treatment resistance, disease recurrence, and severe side effects. In recent years, growing evidence has highlighted the anti-inflammatory, antioxidant, and immunomodulatory properties of molecular hydrogen (H_2). Due to this, its potential application in oncology is of particular interest.
Aim. To investigate the effect of molecular hydrogen on the tumour microenvironment (inflammatory processes, immune infiltration, and proliferative activity) in an experimental model of colorectal cancer.
Methods. The study was conducted on 90 male Wistar rats. CRC was induced by weekly subcutaneous injections of 1,2-dimethylhydrazine for 30 weeks. Animals were divided into three groups: intact control, CRC without correction, and CRC with continuous access to hydrogen-enriched water (0.6 ppm, generated using magnesium sticks). On day 211, animals were euthanized, and colon tissue was examined by immunohistochemistry for CD4+, CD8+, CD20+, CD68+ cell subpopulations, TNF-α, Ki-67, and p53 expression.
Results. In untreated CRC animals, a pronounced immune and inflammatory response was observed: the colonic wall showed massive infiltration by T-helper cells (CD4+), B-lymphocytes (CD20+), and macrophages (CD68+), with TNF-α levels increasing dozens of times. Cytotoxic T-lymphocytes (CD8+) also increased, but to a much lesser extent, resulting in a high CD4+/CD8+ ratio indicative of predominant humoral immunity and relatively weak cytotoxic activity. Proliferative activity of tumour cells (Ki-67) was markedly elevated, and p53 expression increased 6–7-fold, most likely reflecting accumulation of mutant forms of the protein. Animals that consumed hydrogen-enriched water throughout the entire carcinogenesis period demonstrated noticeable positive changes. The anti-inflammatory effect was the most prominent, as evidenced by a decrease in CD68+ cell infiltration and TNF-α levels (18–25% less) compared to the untreated CRC group. Simultaneously, immune infiltration was remodelled – the numbers of T-helper cells and B-lymphocytes slightly decreased, whereas cytotoxic CD8+ lymphocytes increased substantially (by nearly 72%), shifting the balance toward enhanced cell-mediated antitumour immunity. However, molecular hydrogen had no effect on tumour cell proliferative activity (Ki-67 remained equally high) or p53 expression, which stayed unchanged as a marker of genetic instability and tumour progression.
Conclusions. Long-term administration of hydrogen-enriched water in a rat model of CRC exerts pronounced anti-inflammatory and immunomodulatory effects and promotes reorientation of the immune response from humoral to more effective cytotoxic immunity. Thus, H_2 can be regarded as a safe and promising adjuvant that improves the tumour microenvironment, attenuates the inflammatory component, and enhances the organism's natural cytotoxic antitumour response.
Source(s) of research support: This research was conducted without any external funding.
Ethical Committee Approval: The TNMU Bioethics Committee (Protocol No. 41, June 1, 2017).
Acknowledgments: We thank Cedars-Sinai Medical Center's International Research and Innovation in Medicine Program and the Regional Cooperation for Health, Science, and Technology Association (RECOOP HST Association) for their support.

Cell Encapsulation for Wound Healing: Storage and Transportation of Mesenchymal Stem Cells at Ambient Temperature

Petrenko O.[1,2], Trufanova N.[1], Cherkashina D.[1], Bozok G.[1], Pakhomov O.[1], Revenko O.[1]

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Keywords: mesenchymal stromal/stem cells, storage, ambient temperature, 3D constructs, tissue engineering

Introduction: Human mesenchymal stromal/stem cells (MSCs) possess unique proliferative, differentiation, and secretory potentials, which are realized more effectively in 3D MSC-based constructs. Ambient temperature preservation can be a safer, easier, and more cost-effective short-term storage and transportation method. 3D constructs support more natural MSC organization and can induce metabolic reconfiguration, resulting in increased resistance to ambient storage conditions.
Aim: To study the metabolic mode and functional properties of MSCs before and during storage at ambient temperature in suspension and alginate microspheres (AMSs).
Methods: Human adipose tissue-derived MSCs were isolated from lipoaspirate samples (informed consent, collagenase digestion). AMS were generated via electrospraying. MSCs in suspension and within AMSs were cultured (alpha-MEM, 10% FBS, 50 μg/mL penicillin/streptomycin) for 3 days, then stored in culture medium within sealed cryotubes (Nunc) at 22 °C. Viability/apoptosis (6-CFDA/annexin V-Cy3 or FDA/EB), metabolic activity (Alamar Blue), actin filaments (Phalloidin-FITC), and differentiation potential were assessed before and after storage. Reactive oxygen species (ROS) levels (DCFH-DA) were analyzed during storage. Wound healing efficiency was determined planimetrically and histologically in full-thickness wound model in mice.
Results: Metabolic activity of MSCs decreased after encapsulation and accompanied by F-actin reduction without affecting cell viability. Low level of metabolic activity of cells kept during culturing. After 3 days of storage at ambient temperature MSCs within AMSs maintained viability, proliferation, and differentiation, unlike in suspension cultures. During storage number of annexin-positive cells and ROS production remained unchanged in AMSs and increased significantly in suspension. Application of MSCs within AMCs to the surface of a full-thickness cutaneous wound in mice, complicated by Mitomycin C, led to accelerated epithelialization and wound closure. The wound-healing property of the alginate-encapsulated cells was maintained after storage at ambient temperatures for at least 3 days.
Conclusion: Therefore, encapsulation in alginate hydrogel provides metabolic reconfiguration and enhances MSC resistance to ambient storage damage, which accelerates wound healing.
Ethical Committee Approval: The Institute's bioethics committee, 14.04.2016, protocol 2.
Financial Support: This study was supported by National Research Foundation of Ukraine (project № 2021.01/0276).
Acknowledgement: We thank Cedars-Sinai Medical Center's International Research and Innovation in Medicine Program and the Regional Cooperation for Health, Science and Technology Association (RECOOP HST Association) for their support of our study and our organization as a participating Cedars-Sinai Medical Center – RECOOP Research Center (CRRC).

Modulation of Calreticulin by the Thiazolidinone Derivative Les-6650 as a Therapeutic Strategy for Myeloproliferative Neoplasms

Finiuk N.S.[1,2], Ivasechko I.I.[1,2], Kozak Yu.S.[1,2], Klishch M.V.[2], Yushyn I.M.[3], Manko N.O.[1], Klyuchivska O. Yu.[1], Lesyk R.B.[2,3], Stoika R.S.[1]

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[2] Molecular Design Center, State Danylo Halytsky Lviv National Medical University, Lviv, Ukraine
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Keywords: calreticulin (CALR), myeloproliferative neoplasms (MPN), cytotoxicity, affinity towards CALR

Introduction: *CALR* mutations are critical drivers of MPN development, acting as gain-of-function alterations that trigger overproduction of blood cells. By forming a neoantigen that abnormally activates the thrombopoietin receptor, these mutations cause unchecked cell growth. This mutant protein in transformed cells is a promising target for novel chemotherapies.
Aim: We aimed to develop CALR modulators for use in MPN therapies.
Methods: AutoDock Vina 1.2.3 and GROMACS 2025.1 software were used for an *in silico* study. The toxicity of compounds was explored with MTT. Diphenylamine assay, Methyl green replacement assay, and Western-blot analysis were used to investigate the mechanism of action.
Results: Novel heterocyclic small molecules based on non-condensed and condensed thiazolidinone derivatives were synthesized. We found that derivative Les-6650 exhibited selective activity toward MPN model cells, including Ba/F3 MPL CALR del 52 and Ba/F3 MPL CALR ins5 harboring *CALR* ins5 and *CALR* del52 mutations. This compound did not exhibit significant toxicity towards acute T-cell leukemia cells, human breast, colon, lung, hepatocytic, glioblastoma cells, as well as towards lymphocytes and granulocytes of healthy donors (IC_{50} > 50 μM). The affinity of compound Les-6650 for the N-domain of wild-type CALR was -6.254 ± 0.234 kcal/mol, the most favorable docking poses of Les-6650 yielded scores of -6.841 and -6.737 kcal/mol. Results of molecular dynamics simulations, performed in triplicate over 10 ns, showed that the predicted interaction energy of CALR with Les-6650 was -241.06 ± 19.07 kJ/mol. This compound increased the level of wild-type CALR protein in Ba/F3 MPL CALR ins5 and Ba/F3 MPL CALR del52 cells. No increase in phosphorylated Jak2, STAT5 levels was observed. Les-6650 induced DNA fragmentation and apoptosis in Ba/F3 with CALR mutations. Les-6650 (30-120 mg/kg) demonstrated a favorable safety profile, characterized by minimal changes in body weight (≤6%), stable leukopoiesis and erythropoiesis, and normal organ-to-body weight ratio.
Conclusion: The obtained data indicate the potential of the Les-6650 compound as a promising candidate for targeting MPN cells carrying CALR mutations.
Sources of research support: The research was funded by a grant from the National Research Foundation of Ukraine (project No. 2023.03/0104).
Ethical Committee Approval: All animal experiments were approved by the Bioethics Committee of the Institute of Cell Biology of the NAS of Ukraine (Protocol No. 2025-1, 05 February 2025).
Acknowledgements: The authors thank Dr. Robert Kralovics (Medical University of Vienna, Austria) for providing the cells. We also thank Cedars-Sinai Medical Center's International Research and Innovation in Medicine Program, and the Regional Cooperation for Health, Science and Technology Association (RECOOP HST Association) for their support.

Antifibrotic Activity of Juglone-Based Thiopyrano[2,3-D]Thiazole in TGF-β1-Stimulated Fibroblasts Model

Ivasechko I.[1,2], Lozynskyi A.[3], Kozak Yu.[1,2], Finiuk N.[1,2], Manko N.[1], Lesyk R[2,3], Stoika R.[1]

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Keywords: fibrosis, transforming growth factor beta 1, thiopyrano[2,3-d]thiazole, fibroblasts model

Introduction: Fibrosis is characterized by an excessive deposition of extracellular matrix (ECM) components, primarily collagen, leading to tissue scarring and organ failure. Transforming growth factor beta 1 (TGF-β1) is the systemic profibrotic cytokine that drives fibroblast-to-myofibroblast differentiation, marked by α-smooth muscle actin expression and enhanced ECM synthesis.
Aim: Here, we report about anti-fibrotic effect of novel thiopyrano[2,3-d]thiazole derivative including 5-hydroxy-1,4-naphthoquinone (juglone) scaffold that is known for its redox and anti-inflammatory properties.
Materials and Methods: BALB/3T3 mouse embryonic fibroblasts and BJ human fibroblasts were cultured in DMEM supplemented with 1 % fetal bovine serum. Fibrotic phenotype was induced in two models via stimulation with recombinant human TGF-β1 (2 ng/mL) for 48 h; or exposure to hypoxia (2%) for 24 h.
The tested compound (thiopyrano[2,3-d]thiazole–juglone hybrid) was added simultaneously with the pro-fibrotic stimulus or 24 h later in 0.1–10 μM concentrations. Cell viability was assessed using MTT assay. Collagen deposition was quantified by Sirius Red staining (absorbance at 540 nm after elution in 0.1 N NaOH) and by measurement of hydroxyproline content using a colorimetric hydroxyproline assay kit. Myofibroblast differentiation and intracellular protein expression were evaluated by the Western blot analysis. Morphological changes and actin cytoskeleton reorganization were visualized by fluorescence microscopy.
Results: New thiopyrano[2,3-d]thiazole in 0.1-0.5 μM doses reduced Sirius Red staining in TGF-β1 stimulated fibroblasts, while maintaining high cell viability. Higher dose (10 μM) of this compound reduced the viability of these cells. Taken microphotographs revealed fewer elongated, stress fiber-rich myofibroblasts in the treated group.
Conclusions: Thiopyrano[2,3-d]thiazole in low concentrations exhibits potent antifibrotic effects *in vitro* by suppressing TGF-β1-mediated collagen synthesis and myofibroblast differentiation without compromising fibroblast viability.
Sources of research support: This work was supported by a grant №0125U002857 for Young Scientists of the National Academy of Sciences of Ukraine (2025–2026).
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Nongenomic Effects of 17β Estradiol on Gastric Smooth Muscle: Sex Difference and Signaling Mechanism in Rats

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Keywords: smooth muscle, relaxation, estradiol, stomach, fundus, sex

Introduction: 17β Estradiol (E2) exerts its classic effects through slow, genomic pathways. However, rapid, nongenomic effects of E2 are increasingly explored on different tissues, including smooth muscle. The role and mechanism of these nongenomic effects in regulating gastric fundus motility, as well as the gender-dependent effect, remain poorly understood.
Aims: This study aimed to investigate the rapid, nongenomic effects of E2 on the gastric fundus smooth muscle in male and female rats and to characterize the receptor pathways involved.
Methods: Isolated gastric fundus longitudinal muscle (FLM) and circular muscle (FCM) strips from male and female rats were pre-contracted with 40 mM KCl using an in vitro experimental setup to quantify changes in contractile responses. The effect of E2 was tested in cumulative concentrations (10^{-8} - 10^{-3} M). To elucidate signaling mechanisms, tissues were preincubated with fulvestrant (a classical ER antagonist), mifepristone (a progesterone and glucocorticoid receptor antagonist with affinity for membrane ERs), or G15 (a G protein-coupled estrogen receptor (GPER) antagonist), all at 10^{-6}M.
Results: E2 produced potent, concentration-dependent relaxation in KCl-precontracted fundus strips of both sexes, with Emax of 56.5% (FLM) and 61.0% (FCM) in males, and 55.3% (FLM) and 63.4% (FCM) in females. However, E2 showed higher potency in females, with EC50 values of $2.3x10^{-5}$ M (FLM) and $4.3x10^{-5}$ M (FCM), compared to $4.9x10^{-5}$ M (FLM) and $7.4x10^{-5}$ M (FCM) in males. Pharmacological inhibition revealed that this relaxation was significantly attenuated by mifepristone and G15, but not by fulvestrant.
Conclusion: These findings highlight a novel, sex-specific, nongenomic mechanism for estrogenic regulation of gastric motility, in which E2 produces a more potent relaxation effect in female rats. This effect is mediated through a pathway involving both mifepristone-sensitive receptors and G-protein estrogen receptors.

Source(s) of research support: This study was supported by a Stipendium Hungaricum scholarship.
Ethical Committee Approval: All experiments involving animal subjects were carried out with the approval of the National Scientific Ethical Committee on Animal Experimentation (permission number XIII/72/2020).
Acknowledgment: We thank Cedars-Sinai Medical Center's International Research in Medicine Program and the Regional Cooperation for Health, Science, and Technology Association (RECOOP HST Association) for their support and the participating Cedars-Sinai Medical Center - RECOOP Research Centers (CRRCs).

Smooth Muscle Electromyography for Assessing Gastroparesis Induced by Vagotomy, Dexamethasone, and Pantoprazole in Rats

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Keywords: gastroparesis, vagotomy, dexamethasone, pantoprazole, electromyography

Introduction: Gastroparesis is a gastrointestinal motility disorder characterized by delayed gastric emptying without mechanical obstruction. Developing reliable experimental models is crucial for understanding the mechanisms of disease and identifying effective therapeutic interventions.
Aim: Our aim was to establish three rat models of gastroparesis and to assess gastrointestinal myoelectrical activity (MA) across all GI segments using in vivo smooth muscle electromyography (SMEMG).
Methods: Male Sprague-Dawley rats (250–350 g; n=12/group) were allocated to vagotomy or drug-induced gastroparetic models. Gastric and intestinal MA was recorded via SMEMG. The vagotomy group underwent subdiaphragmatic truncal vagotomy with weekly recordings for 8 weeks. An acute neostigmine (0.8 mg/kg, i.v. bolus) was administered in the vagotomy group at week 2, with recordings taken pre- and post-injections. Pharmacological groups received daily intraperitoneal injections for 6 days (dexamethasone 1 mg/kg, pantoprazole 10 mg/kg, or vehicle), with recordings on day 7. An additional group received dexamethasone 10 mg/kg orally for three days, with recordings on day 4.
Results: SMEMG across three distinct gastroparesis models demonstrated significant suppression of gastric MA in all experimental groups**.** Subdiaphragmatic vagotomy induced a progressive decline, culminating in a 66.2% reduction by week 7 ($p<0.0001$). Acute neostigmine administration at week 2 post-vagotomy significantly reversed the MA, reducing it from 50% to 32.7% below control levels ($p<0.05$). Small-intestinal MA was minimally affected (with a maximum of 17.6% reduction), whereas large-intestinal MA showed severe, progressive suppression, reaching 71.4% by week 7 ($p<0.0001$).
Pharmacologically, dexamethasone elicited opposite effects on motility: intraperitoneal administration (1 mg/kg for 6 days) reduced gastric MA by approximately 30%, whereas oral administration (10 mg/kg for 3 days) stimulated gastric MA by 50% and small-intestinal MA by 35%, with no significant effect on large-intestinal MA. Pantoprazole (10 mg/kg i.p.) consistently suppressed gastric MA, reducing it by 35% after 6 days and 39% after 3 days
Conclusion: This study establishes three rat models of gastroparesis with distinct MAs. Our findings underscore their value of SMEMG as a tool for investigating region-specific gastrointestinal pathophysiology and for testing novel prokinetic agents preclinically.
Source of research support: This study was supported by a Stipendium Hungaricum scholarship.
Ethical Committee Approval: All experiments involving animal subjects were carried out with the approval of the National Scientific Ethical Committee on Animal Experimentation (permission number XIII./735/2023).
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Effects of Dextromethorphan on Neurotransmission in Rat Synaptosome

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Keywords: dextromethorphan, glutamate release, monoamine uptake, monoamine oxidase activity

Introduction: N-methyl-D-aspartate receptors (NMDA-Rs) play a central role in synaptic plasticity. Rapid antidepressant effect of ketamine, an NMDA-R antagonist, has recently gained substantial attention. Another NMDA antagonist, dextromethorphan (DM), has also emerged as a compound of interest due to its antidepressant potential. Because several effects of DM were reported, a detailed characterization of its dose-dependent pharmacodynamic actions is of considerable significance.
Aims: Our objective was to examine how DM influences glutamatergic and monoaminergic neurotransmission. Furthermore, we sought to characterize its dose-dependent actions and to elucidate potential molecular mechanisms underlying its psychopharmacological effects.
Methods: Synaptosomal glutamate release was quantified by an enzyme-coupled fluorescence assay. To evaluate monoamine transport, we applied its fluorescent substrate. Its monoamine oxidase (MAO) inhibitory effect was assayed by using fluorescence-based detection method. Concentrations were chosen according to the literature.
Results: At low DM concentrations (1 nM–10 μM), we observed a significant elevation in glutamate release, indicating enhanced synaptic activity. At higher concentration (100 μM), glutamate release returned to baseline levels. DM at 400 and 800 μM markedly reduced the monoamine uptake, whereas lower concentrations produced no significant change. Our investigation also identified a previously unrecognized functional component; DM inhibited both MAO isoforms weakly, yet even at a concentration of 10 μM the inhibition reached statistical significance.
Conclusion: Our findings demonstrate that DM modulates neurotransmission in a dose-dependent fashion, potentially via differential inhibition of NMDA-R subtypes. The concentration-dependent reduction in monoamine uptake and MAO activity indicates that DM influences multiple neurotransmitter systems. Together, these results refine our understanding of its pharmacological profile.

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Ethical Committee Approval: -
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Cell Death and DNA Damage in Human Esophageal Carcinoma Cells Exposed to Low-Energy Protons

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Keywords: low-energy protons; carcinoma cells; DNA damage; caspase-3

Introduction: Proton radiation dominates galactic cosmic rays and solar particle events. As a result, it represents serious health risks for astronauts during prolonged space missions and for aircrew exposed to high-altitude flights. High-energy protons are also used in clinical practice for proton therapy, and DNA double-strand break induction by them has therefore been studied extensively. Much less is known about the effects of low-energy protons (≤12 MeV).
Aim: The aim of our study was to explore the efficacy of low-energy proton radiation to induce DNA damage and cell death.
Methods: SRIM (Stopping and Range of Ions in Matter) simulations of proton ranges were used to set the experimental geometry. Monolayers of human esophageal carcinoma Kyse 450 cells were irradiated by proton beams with energies of 1.5, 3.0 and 5.4 MeV for 1–100 s, or with UVC light for 15 min. Modified clonogenic survival tests were used to determine irradiation conditions suitable for subsequent analyses. The kinetics of caspase-3/7 activation and loss of membrane integrity were monitored at several time points using CellEvent™ Caspase-3/7 and propidium iodide staining. DNA double-strand breaks were detected by γ-H2AX immunofluorescence.
Results: SRIM simulations showed that increasing proton energy from 1.5 MeV to 5.4 MeV shifted the Bragg peak position, representing maximum energy deposition, from 2.5 cm to 37.6 cm, respectively. Proton irradiation with energy of 5.4 MeV for 50 s has been selected for all subsequent experiments. Caspase-3/7 activation followed a sigmoidal time-course for both radiation types, with an earlier half-maximum response after proton irradiation than after UVC (5.4 h vs. 11.9 h), indicating earlier caspase 3/7 activation for protons. Propidium iodide fluorescence, reflecting loss of membrane integrity, increased with similar kinetics. In cells irradiated by UVC, γ-H2AX foci were observed, whereas pan-nuclear γ-H2AX staining prevailed in cells irradiated by protons. Despite the lack of comparison with clinically used proton energies, the study provides useful insight into health risks at high altitudes and in space.
Conclusions: Low-energy 5.4 MeV protons efficiently killed Kyse 450 cells located at the Bragg peak, with much stronger effects compared to UVC light.
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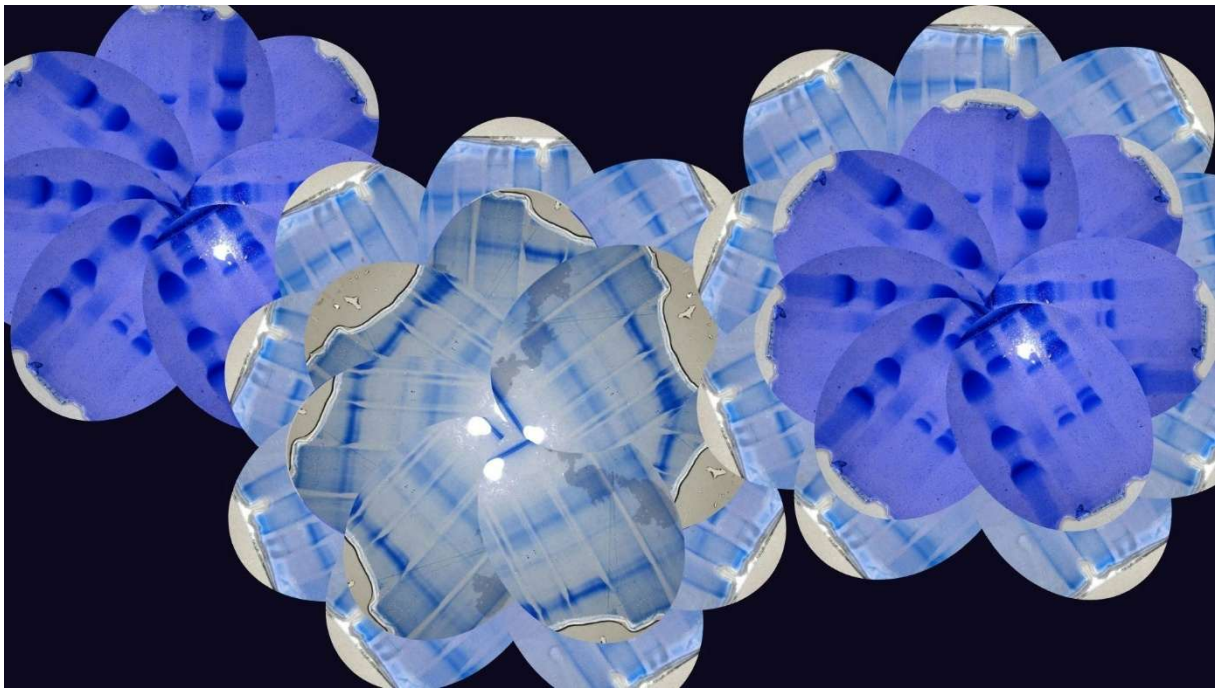
Neurodegenerative Diseases (NDD)



Biology Student's Forget-me-not

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Psychological and Cognitive Consequences of War Experience in Combat Soldiers and Veterans

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Keywords: military personnel, veterans, cognitive impairment, post-traumatic stress disorder

Introduction: Combatants and veterans represent a population with specific psychological, neurological, and social characteristics. Exposure to combat situations significantly increases the risk of cognitive disturbances, including impaired attention, memory decline, reduced processing speed, and deficits in executive functioning. These changes frequently occur in the context of post-traumatic stress disorder (PTSD), mild traumatic brain injury (mTBI), chronic stress, and prolonged physical exhaustion. Timely identification of cognitive impairment is essential for developing effective rehabilitation and support strategies.
Aims: To investigate the structure and severity of cognitive changes in combatants and veterans and to propose approaches for improving diagnosis and rehabilitation based on the detected impairments.
Methods: Following the principles of biomedical ethics, a total of 89 participants (active-duty Combatants and veterans) with documented combat experience were examined. Cognitive functioning was assessed using the Montreal Cognitive Assessment (MoCA), the Trail Making Test (A/B), and the Digit Span Test. PTSD symptoms were evaluated using the PCL-5 scale/ The condition was assessed through a clinical interview and psychodiagnostic examination (HADS depression and HARS anxiety scales, Symbolic Communication Test (TMT-A and TMT-B), Mississippi Posttraumatic Stress Disorder Scale, and Quality of Life assessment methodology in adapting N. O. Maruta. Clinical interviews and structured questionnaires were also used to assess emotional state and psychosocial functioning. Statistical analysis was performed using SPSS 26.0.
Results: Cognitive dysfunction was observed in a substantial proportion of participants, primarily affecting attention control, working memory, and executive processes. Veterans demonstrated more pronounced deficits than active-duty personnel. PTSD severity showed a moderate correlation with impaired cognitive flexibility, while the history of mild TBI was associated with a significant decrease in overall cognitive performance. The results highlight the polymorphic nature of cognitive changes, which are intertwined with emotional disturbances and stress-related psychopathological symptoms.
Conclusions: Cognitive impairments in combatants and veterans are strongly influenced by PTSD, combat exposure, and mild TBI. Improving diagnostic protocols and implementing targeted cognitive-rehabilitation programs can significantly enhance functional recovery and social reintegration. The findings support the need to incorporate cognitive assessment into routine clinical practice for this population.
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Ethical Committee Approval: Protocol № 83 from Nov 3, 2025, of Bioethics Commission of the I. Horbachevsky Ternopil National Medical University.
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Comparative Characteristics of Psychological Mechanisms of Resilience and Stress Tolerance in Combatants and Veterans

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Keywords: combatants, veterans, resilience, stress tolerance, psychological adaptation

Introduction. Combatants and veterans are exposed to long-term, high-intensity stressors, including life-threatening situations, chronic uncertainty, physical overload, and the emotional consequences of combat. Under these conditions, specific psychological mechanisms of resilience and stress tolerance are formed—key constructs that determine mental stability, adaptability, and long-term functional outcomes. High resilience acts as a protective factor, reducing the risks of anxiety, depression, PTSD symptoms, and maladaptive behaviours. In contrast, reduced stress tolerance increases vulnerability to psychological exhaustion and deterioration. Studying resilience mechanisms is essential for developing effective preventive and rehabilitation programs.

Aim. To examine the levels, structural components, and psychological mechanisms of resilience and stress tolerance in combatants and veterans, and to identify predictors of adaptive and maladaptive responses to prolonged combat-related stress.

Methods. In accordance with biomedical ethics principles, 163 participants (active-duty combatants and veterans) with verified combat experience who were undergoing treatment at the Ternopil Regional Communal Psychoneurological Hospital were examined. Resilience was assessed using the Connor–Davidson Resilience Scale (CD-RISC-25). Stress tolerance and coping behaviour were evaluated using the Brief Hardiness Scale and the Multidimensional Scale of Perceived Social Support (MSPSS). Additional structured clinical interviews and questionnaires were administered to assess emotional state, psychosocial functioning, and personality characteristics. Statistical analysis was performed using SPSS 26.0.

Results showed that he obtained data demonstrating substantial interindividual variability in resilience mechanisms. Veterans showed lower stress tolerance compared with active-duty combatants, which is associated with accumulated traumatic exposure and chronic post-combat stress. High resilience scores were associated with lower perceived stress, reduced emotional exhaustion, and more frequent use of adaptive coping strategies such as active problem-solving and cognitive restructuring. Low resilience levels were linked to avoidant behaviour, increased anxiety, depressive symptoms, and impaired social functioning.

Conclusions. The psychological mechanisms of resilience and stress tolerance are key determinants of mental well-being in combatants and veterans. Their enhancement through psychological training, targeted psychoeducation, and supportive interventions can substantially improve adaptation to combat stress, reduce the risk of mental disorders, and optimise psychosocial functioning. Integrating resilience assessment and development programs into the mental-health support system for military personnel is advisable.

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Toxic Effect of Cu Excess in Brain Nerve Terminals and Its Neutralization

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Key words: copper, neurotoxicity, neurotransmitters, neuroprotection, brain nerve terminals

Introduction: Trace metal Cu is dangerous neuropollutant. The intracellular concentration of Cu^{2+} is relatively low, and its increase can lead to cytotoxicity and cell death. Excessive intake and dysregulation of Cu^{2+} homeostasis caused its accumulation in the brain, and Cu^{2+} excess induced apoptosis/cuproptosis in neuronal cells, and the loss of neurons led to memory deficits and cognitive impairment (Nobili et al., 2017). Mitochondrial dysfunction can be involved in Cu^{2+}-induced impairment of synaptic plasticity and cognitive disability (Feng et al., 2023).
Aims: To analyse toxic effect of Cu^{2+} in cortex nerve terminals and ways for its elimination.
Methods: The nerve terminals (synaptosomes) were isolated from Wistar rats. [^{3}H]GABA and L-[^{3}H]glutamate transportation in nerve terminals was monitored using a radiolabelled assay. The fluorimetric experiments were carried out using the dyes JC-1 and DCF.
Results: In nerve terminals, Cu^{2+} (50 μM) increased the extrasynaptosomal level of excitatory and inhibitory neurotransmitters L-[^{3}H]glutamate and [^{3}H]GABA, respectively. In the fluorimetric experiments using the dye JC-1 to measure the mitochondrial potential, a calculated level of the intensity of the fluorescence signal in response to the application of Cu^{2+} (50 μM $CuSO_4$) was 64.70 ± 3.57 % as compared to 100 % in the control. So, Cu^{2+} depolarised the mitochondrial membrane in nerve terminals. ROS generation was monitored using DCF. The addition of Cu^{2+} (50 μM) led to an increase in spontaneous ROS production as compared to control. These fluorimetric data completely corresponded to the results obtained using L-[^{3}H] glutamate and [^{3}H]GABA. Different "green" carbon nanoparticles/materials were effective in adsorption of Cu^{2+} in biological system, such as nerve terminals. In particular, "green" carbon dots from coffee waste, biochars from corn cobs and dry apples were able to reduce toxic effect of Cu^{2+} in nerve terminals.
Conclusions: Cu^{2+} excess induced neurotoxic signs in nerve terminals through increased extrasynaptosomal level of neurotransmitters, disturbance of excitation-inhibition balance, depolarization of the mitochondrial membrane and altered redox state. These toxic effects can be eliminated using unfunctionalized "green" carbon nanoparticles and materials.
Source of research support: This work was supported by the National Research Foundation of Ukraine: NRFU grant # 2023.03/0036 «Functionalized carbon-based nanomaterials obtained by "green methods" from Ukrainian agricultural waste to combat multilevel neurotoxicity induced by xenobiotic/essential transition metals and arsenic».
Ethical approval: The experimental procedures were conducted according to "Scientific Requirements and Research Protocols" and "Research Ethics Committees" from the Declaration of Helsinki. Experimental protocols were approved by the Animal Care and Use Committee of the Palladin Institute of Biochemistry (Protocol # 1 from 10/01/2024).
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Impact of Military Life and Gender on Glycan Age

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Keywords: military life, sex differences, glycan age

Introduction: The lifestyle of professional soldiers, unlike the general population, involves rigorous maintenance of physical fitness and regular meals. Both factors positively influence cardiovascular and metabolic health, which could reflect in glycan age, particularly among premenopausal women.
Aims: To investigate the influence of military lifestyle and gender on glycan age.
Methods: The study included 96 professional soldiers (59 men and 37 woman) and 100 civilians (46 men and 54 women) from Slavonia and Baranja region of Croatia. The International Trauma Questionnaire (ITQ) was used to assess level of stress among participants. Blood samples were collected from all participants using QIAcard Bloodstain Cards (Qiagen, Cat. No. WB100014).
Results: Low levels of stress were recorded in both study groups. The mean chronological age for the control group was 33.9±8.2, while for soldiers it was 31.2±4.4. The mean glycan age for the control group was 39.5± 15.9, and for soldiers, it was 39.1±17.7. The independent t-test did not reveal statistically significant differences in glycan age between soldiers and civilians ($t = 0.14$, $p = 0.886$). Multiple linear regression analysis with glycan age as the dependent variable and chronological age, group membership and gender as predictors showed that male sex was associated with lower glycan age compared with females ($B = 5.61$; $p = 0.021$).
Conclusion: With the condition of equally moderate stress levels between professional soldiers and civilians, the main predictor of glycan age is gender. Despite regular physical training and nutrition, the military lifestyle does not reflect on glycan age.

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Ethical Committee Approval of MFOS (Class: 641-01/24-01/05, Registration Number: 2158-61-46-24-19) from 15.03.2024.
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Ischemia-Induced Proteomic Changes in the Mouse Cortex: Discovery of New Markers for Neural Damage and Repair

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Keywords: brain ischemia, middle cerebral artery occlusion (MCAO), magnetic resonance imaging (MRI), proteomics, gene ontology

Introduction: Stroke remains a major clinical and socioeconomic burden, with limited diagnostic and therapeutic options and a continued need for dependable biomarkers to support patient evaluation and treatment.
Aims: This study investigated proteomic alterations in the cerebral cortex of male WT mice subjected to transient MCAO with chronic hypoperfusion, comparing molecular changes in the acute and chronic phases of ischemic injury.
Methods: Twenty-three mice were allocated into three longitudinal groups: pre-stroke controls, acute post-stroke (24 h), and chronic post-stroke (35 days). Lesion formation, neurological impairment, and functional recovery were verified using MRI and standardized neurological scoring, while cortical tissue from both hemispheres was analyzed by data-independent acquisition mass spectrometry (MS) and SWATH-MS, followed by quantitative and gene ontology-based bioinformatic analysis.
Results: Data-independent acquisition mass spectrometry of cortical tissue identified 74 differentially expressed proteins exhibiting distinct temporal expression profiles. These proteins were categorized into four clusters reflecting acute and chronic upregulation or downregulation. Gene ontology analysis revealed disruptions in pathways related to synaptic function, immune activation, cytoskeletal regulation, and cellular remodeling. Integrating proteomic data with structural and functional outcome measures enabled the identification of 13 proteins not previously linked to stroke pathology, 10 within the ipsilateral cortex (*Dbi, Cpne3, Dnm2, Eef1a1, Taldo1, Pgls, Gnb5, Phf24, Ctsz, Capg*) and 3 within the contralateral cortex (*Agpat3, Cacng8, Endod*).
Conclusion: These results enhance the understanding of molecular processes underlying both injury progression and recovery and highlight novel candidate biomarkers for ischemic brain damage.
Source(s) of research support: KK.01.1.1.07.0071 project "Synergy of molecular markers and multimodal *in vivo* imaging during preclinical assessment of the consequences of ischemic stroke".
Ethical Committee Approval: Ethics Committee and Animal Welfare Committee of the University of Zagreb School of Medicine (approval number 380-59-10106-23-111/25, 23rd February 2023) and the Ministry of Agriculture of the Republic of Croatia (approval number UP/I-322-01/23-01/4, 25th May 2023).
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Heterotypic Neuro-Mesenchymal Spheroids as a Promising 3D Model of Neuroregeneration

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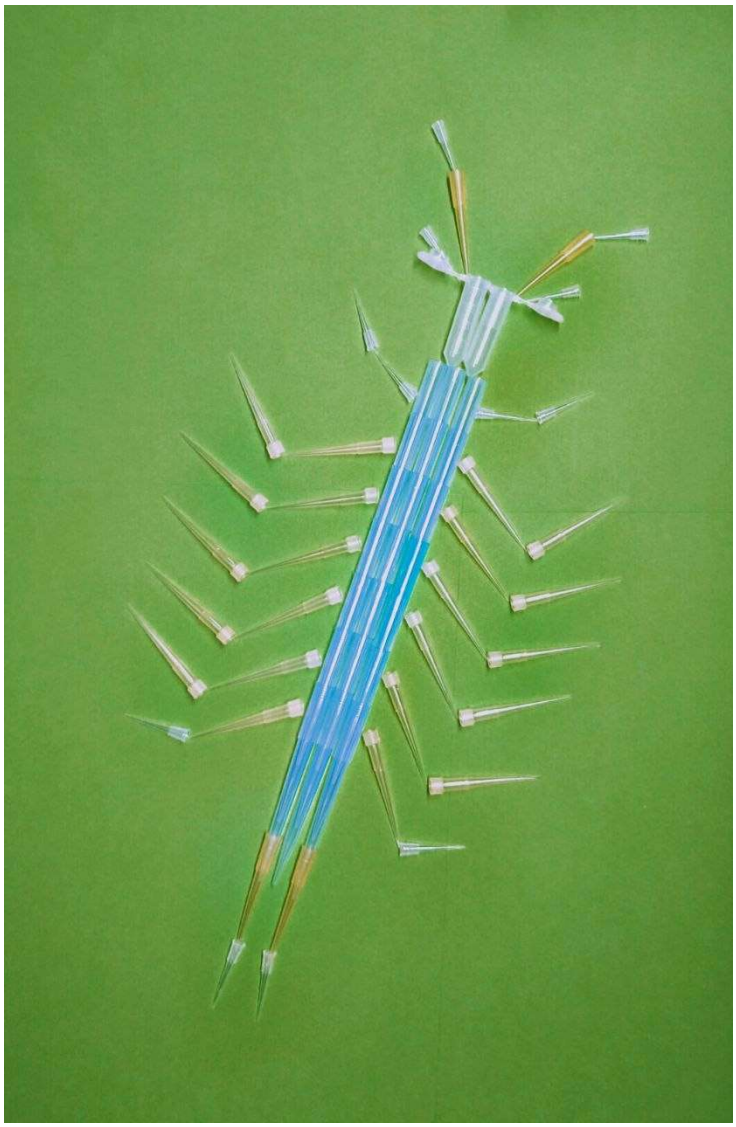
Keywords: neural cells, mesenchymal stem cells, heterotypic spheroids, neuroregeneration

Introduction: The restoration of nervous tissue after injuries and neurodegenerative diseases remains a challenging task due to the low regenerative capacity of mature neurons. Mesenchymal stem cells (MSCs) are one of the most promising agents for cell therapy. To study the mechanisms of their neuroprotective action, the most physiological models are three-dimensional heterotypic models, which reproduce contact and matrix-dependent interactions.
Aims: To determine the optimal conditions for formation and to study the structural and functional characteristics of mixed spheroids composed of neural cells (NCs) (neuroblasts, neural stem/progenitor cells, glial cells) and MSCs.
Methods: NCs were isolated from the brain tissue of newborn rats (P0); MSCs were derived from embryonic rat liver at ED15. For spheroid formation, MSCs (3rd passage) and NCs (2nd passage) were used. Spheroids were generated by the hanging drop method at different ratios of NCs:MSCs. Cell viability was assessed by FDA/PI staining; spatial organization by fluorescent labeling of MSCs (Hoechst 33342).
Results: An NCs:MSCs ratio 1:1 was found to be optimal for stable mixed spheroids formation Within 24 hours, dense, morphologically homogeneous and mechanically stable spheroids formed, with a diameter of 120–180 μm and viability > 90%. In such spheroids, the actual NCs:MSCs ratio was ~3:1 due to strong homotypic adhesion of MSCs, which formed compact isolated clusters, whereas NCs formed the main matrix. This architecture is likely driven by differences in adhesion molecule expression: high levels of N-cadherin in NCs, and predominance of integrins and cadherin-11 in MSCs. Addition of MSCs significantly increased the mechanical and osmotic stability of the spheroids, improved survival of neural cells and accelerated their functional recovery after stress. Heterotypic spheroids significantly surpassed mono-type neural ones in key characteristics: formation efficiency – 95% vs 70%; formation time – 24 h vs 48 h; sphericity – 85% vs 65%.
Conclusion: This original model first demonstrates the role of MSCs as a structural scaffold, which doubles the process of NCs aggregation and significantly increases their viability. The model allows for a comprehensive study of paracrine, contact, and matrix-dependent mechanisms of MSCs action on NCs under physiological conditions, providing a basis for the development of new biotechnological products for neuroregenerative medicine.
Ethical approval: The Institute´s Bioethics Committee, Protocol № 2 from 23/02-2023.
Acknowledgements: We thank Cedars-Sinai Medical Center's International Research and Innovation in Medicine Program and the Regional Cooperation for Health, Science and Technology Association (RECOOP HST Association) for their support of our study and our organization as a participating Cedars-Sinai Medical Center – RECOOP Research Center (CRRC).

Metabolic Disorders (MTD)



Pipette-cenTIPede

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Mapping the Diabetic Gestation: Fetal Cardiac Ultrasound and Maternal–Fetal Characteristics

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Keywords: diabetes mellitus, fetal, heart, ultrasound, diagnostics

Introduction: In diabetes mellitus, cardiac assessment can detect early structural and functional changes, guide risk stratification, optimize treatment, and help prevent heart failure—even as early as fetal life.
Aims: We aimed to examine the morphological factors affecting the developing fetal heart and to investigate the fetal and maternal correlations in pregnancies complicated by diabetes mellitus. We sought to determine whether the severity of myocardial hyperplasia correlates with the occurrence of infant respiratory distress syndrome and cardiac failure.
Materials and Methods: This prospective case-control study investigated pregnancies complicated by diabetes mellitus that resulted in fetal myocardial hyperplasia. Two ultrasound examinations were performed on 84 pregnant women in accordance with the selection criteria (different types of diabetes mellitus and control cases).
The first ultrasound examination was carried out between the 24th and 28th weeks, and the second between the 33rd and 38th weeks of pregnancy. During the examinations, we measured and averaged fetal biometrics, estimated fetal weight, the amount of amniotic fluid, and the thickness of the ventricular walls and interventricular septum. Clinical data related to maternal diabetes symptoms were also collected, and pregnancy outcomes were monitored. These data were compared with those from the control group and with literature reference values.
Results: Regarding the fetal myocardium and interventricular septum, significantly higher thickness values were recorded compared to the control group. Among the infants, 21% were macrosomic, and 16% were born with intrauterine growth restriction.
Conclusions: Based on our findings, more complications occurred in fetuses and infants of obese and diabetic pregnant women than in those of the control group. Therefore, monitoring fetal myocardial parameters in pregnancies affected by maternal glucose metabolism disorders may serve as an effective screening tool for adverse perinatal outcomes.

Funding: University of Szeged, Albert Szentgyörgyi Medical School, Faculty Research Grant, Géza Hetényi Fund (No: 5S 724 (A202)
Ethical Committee Approval: Regional Ethical Committee Approval (reference number: 327/2015-SZTE).
Acknowledgements:
We thank Cedars-Sinai Medical Center's International Research and Innovation in Medicine Program, and the Regional Cooperation for Health, Science and Technology Association (RECOOP HST Association) for their support to present our results in the 21st RECOOP Bridges in Life Sciences Conference.

Modulatory Effect of Molecular Hydrogen on Oxidative Stress, Glycemic and Lipid Profiles in Metabolic Syndrome

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Keywords: metabolic syndrome, molecular hydrogen, oxidative stress, glycemic profile, lipid metabolism

Introduction. Metabolic syndrome (MS) is a multifactorial condition characterized by obesity, insulin resistance, dyslipidemia, and arterial hypertension. A key component of its pathogenesis is oxidative stress, which leads to damage of lipids, proteins, and DNA. In recent years, molecular hydrogen has attracted attention as a selective antioxidant capable of influencing metabolic processes and adaptive responses of the organism.

Aim. To study the effect of molecular hydrogen on oxidative stress markers, carbohydrate, and lipid metabolism in rats with experimentally induced MS.

Methods. MS was induced in Wistar rats by long-term (6, 12, and 20 weeks) feeding with a high-calorie diet. A subset of rats received water enriched with molecular hydrogen (0.6 ppm). Serum levels of 8-isoprostanes, protein carbonyl, 8-hydroxydeoxyguanosine (8-OHdG), glucose, insulin, HOMA-IR, fructosamine, glycated hemoglobin, triglycerides (TAG), total cholesterol, LDL, and HDL were measured. The efficacy of the intervention was evaluated at 6, 12, and 20 weeks.

Results. Rats with MS exhibited progressive metabolic disturbances. At week 20, oxidative stress markers exceeded control values: 8-isoprostanes by 2.7-fold, protein carbonyl by 2.3-fold, and 8-OHdG by 2-fold. Hyperglycemia increased by 76 % versus control; insulin rose by 55 % at week 6 and 1.7-fold at week 20; HOMA-IR increased correspondingly. TAG increased by 36 % (6 weeks), 47 % (12 weeks), and 64 % (20 weeks); total cholesterol by 57 %, 115 %, and 117 %; LDL by 62 %, 145 %, and 133 %; HDL decreased by 13–28 %.

Consumption of hydrogen-enriched water significantly reduced oxidative stress markers (8-isoprostanes by 30 %, protein carbonyl by 25 %, 8-OHdG by 32 %), mitigated hyperglycemia and insulin resistance (glucose by 32 %, insulin by 24 %, HOMA-IR by 49 %, HbA1c by 28 % at week 20), and improved atherogenic lipid profile (TAG by 28 %, cholesterol by 23 %, LDL-C by 29 %). Effects were minimal at early stages and most pronounced at middle and late stages. Values did not reach control levels, indicating a partial but stable protective effect of molecular hydrogen and its potential in comprehensive MS therapy.

Conclusions. MS is associated with complex metabolic disturbances, including elevated oxidative stress, carbohydrate imbalance, and lipid profile disorders. Molecular hydrogen reduces oxidative stress markers, partially normalizes glycemic parameters, attenuates insulin resistance, and improves the atherogenic lipid profile over prolonged use. These findings support the potential of molecular hydrogen as an adjunct in the comprehensive therapy of metabolic syndrome, warranting further investigation of its mechanisms of action.

Source(s) of research support: This research was conducted without any external funding.

Ethical Committee Approval: The TNMU Bioethics Committee (Protocol No. 41, June 1, 2017).

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Inhibition of Ganglioside Degradation Affects Insulin Signalization and SH-SY5Y Cell Morphology

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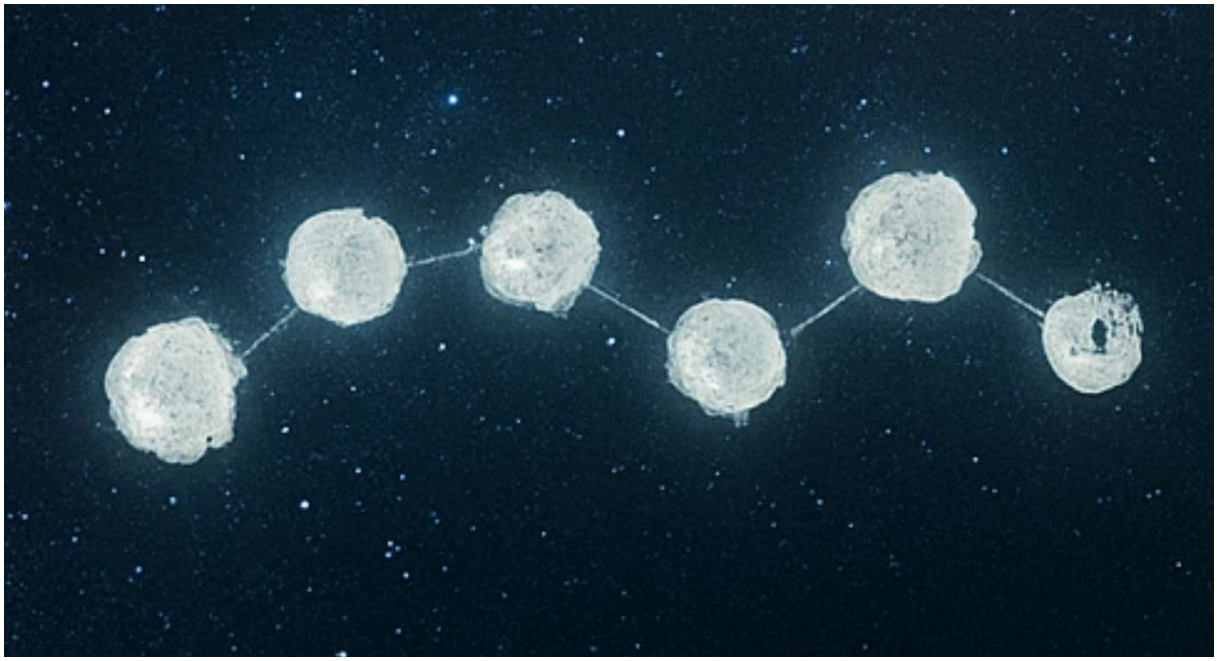
Keywords: gangliosides, insulin receptor, signal transduction, neurites

Introduction: The cell membrane is a key site of intercellular communication, hosting transmembrane receptors that regulate metabolism and energy. Gangliosides, glycosphingolipids, modulate membrane charge and stabilize receptors, including the insulin receptor, crucial for glucose homeostasis and cell metabolism.
Aims: This study examined how inhibiting ganglioside degradation affects insulin signaling and cell morphology.
Methods: SH-SY5Y cells were cultured, differentiated with 10 μM all-trans retinoic acid for 9 days, serum-starved for 24 hours, then treated for 48 hours with conduritol B epoxide (CBE), a ganglioside degradation inhibitor, at 2.5 μM, 10 μM, and 40 μM or solvent control. Half the samples were exposed to insulin for 1 hour before collection. Morphology was assessed by FITC staining after fixation; protein expression levels were analyzed using Western blot.
Results: At 40 μM CBE plus insulin, neurite growth is significantly decreased (0 μM+insulin: 22.19 μm; 40 μM+insulin: 13.40 μm), with a similar but nonsignificant trend without insulin. Western blot results are represented as a relative signal ratio to the untreated control group. AKT levels showed no significant change in control groups (0 μM: 0.99±0.13; 0 μM+insulin: 0.93±0.08), but significantly increased at higher CBE doses, plateauing at 1.16±0.11. pAKT levels rose after insulin challenge as expected but dropped significantly with 40 μM CBE treatment to control levels. GSK3α phosphorylation was unaffected; however, GSK3β phosphorylation at Ser9, indicating inactivation, significantly increased at 40 μM CBE with insulin (0 μM+insulin: 0.73±0.12; 40 μM+insulin: 0.98±0.15). Insulin receptor (IR) levels significantly increased with insulin when treated with 10 and 40 μM CBE, though CBE alone showed a trend toward IR reduction.
Conclusions: Inhibiting ganglioside degradation with CBE disrupts insulin signaling under insulin challenge, causing reduced neurite growth, downstream pathway inhibition, and compensatory IR upregulation.

Source(s) of research support: HRZZ IP 09-2014-2324; NPOO.C3.2.R3-I1.04.0089
Ethical approval: was not required as the research was performed on cell culture analysis only.
Acknowledgements: I thank Prof. Heffer and Prof. Mitrečić for their guidance and support. We thank Cedars-Sinai Medical Center's International Research and Innovation in Medicine Program, and the Regional Cooperation for Health, Science and Technology Association (RECOOP HST Association) for their support.

Cardiovascular Diseases (CVD)



The constellation "KolMatEk"

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The Prognostic Value of Arginase II Determination in Patients with Arterial Hypertension

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Keywords: arterial hypertension, arginase II

Introduction: Despite numerous clinical and experimental studies investigating the role of arginase II in the pathogenesis of cardiovascular damage, there is still no clinical standard for assessing arginase activity or arginase II expression levels in blood or tissues for the diagnosis of cardiovascular diseases.
Aims: This study aimed to determine the prognostic value of arginase II determination in patients with arterial hypertension.
Methods: A total of 76 overweight or obese patients with a confirmed diagnosis of arterial hypertension, who were receiving antihypertensive therapy, were examined in a randomized manner. Patients were divided into two groups: Group 1: 38 patients who achieved the target blood pressure; Group 2: 38 patients who did not achieve the target blood pressure. All participants underwent medical and life history assessment, a general physical examination with blood pressure measurement and body mass index calculation, routine laboratory tests (complete blood count, coagulation profile, biochemical analysis, lipid profile), and immunoassays to determine serum arginase II levels using ELISA kit reagents. Statistical analysis was performed using GraphPad Prism 8.0.1 with parametric and non-parametric tests; $p < 0.05$ was considered statistically significant. ROC analysis was performed to determine the threshold value of arginase II for predicting uncontrolled hypertension.
Results Patients who achieved the target blood pressure had significantly lower serum arginase II concentrations. Based on the ROC analysis, the optimal threshold value of arginase II for predicting uncontrolled hypertension was 0.70 pg/mL (AUC = 0.691 (95 % CI 0.575-0.792), $p < 0.01$, sensitivity 86.8 %, specificity 55.3 %).
Conclusions: According to the ROC analysis results, determining serum arginase II levels in patients with arterial hypertension may serve as an informative marker for stratifying the risk of developing uncontrolled arterial hypertension.

Source(s) of research support: RECOOP – CSMC Fusion Research Grant 2022 #31.
Ethical Committee Approval of the DH LNMU, protocol №8 from 09.26.2022.
Acknowledgments: The study was supported by the Regional Cooperation for of Health, Science and Technology Association (RECOOP HST Association) and the participating Cedars-Sinai Medical Center - RECOOP Research Centers (CRRCs).

Thrombosis Predisposition in PTSD Patients

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Keywords: PTSD, thrombosis, hemostasis, fibrinogen, soluble fibrin

Introduction: It is well established that patients with post-traumatic stress disorder (PTSD) have an elevated risk of cardiovascular disease (CVD), even many years after exposure to the triggering events. Such thrombotic complications as stroke and ischemic heart disease contribute to the markedly increased mortality observed in patients with PTSD. Despite these findings, markers of blood coagulation disorders are rarely assessed in patients with PTSD, creating a long-term risk to their health and survival.
Aims: Therefore, the aim of this study was to analyze the markers of intravascular thrombus formation in PTSD patients.
Methods: Blood plasma of patients with diagnosed PTSD were collected in MNE "Ternopil Regional Psychoneurological Hospital. The Mississippi PTSD Scale was used for dividing the patients according to the severity of PTSD (mild, moderate, severe). Fibrinogen was measured by spectrophotometry with the use of thrombin-like enzyme. Prothrombin time and protein C were measured using standard methods. Pairs of monoclonal antibodies III-3B & II-4d; I-3C & II-4d were used for sandwich ELISA detection of D-dimer, Soluble Fibrin, respectively.
Results: Fibrinogen levels were elevated in all patients with PTSD compared with the reference group. Soluble fibrin, which reflects intravascular thrombin generation, was increased in some patients across all study groups. A decrease in protein C levels, indicating its consumption during anticoagulant activity, was observed in the severe PTSD group (75% versus 100% in the reference group). In addition, D-dimer concentrations were elevated in all patients; however, the increase was more pronounced in the moderate and severe groups compared with the mild group (280 ng/mL in the mild group; 704 ng/mL in the moderate group; and 513 ng/mL in the severe group).
Conclusion: Most patients with PTSD demonstrated a pronounced predisposition to thrombosis, as reflected by elevated fibrinogen levels, reduced protein C activity, and increased concentrations of soluble fibrin and D-dimer. These parameters correlated with the severity of PTSD, indicating a higher risk of thrombotic complications in patients with more severe forms of the disorder.

Source of research support: NAS of Ukraine, 0124U000251.
Ethical Committee Approval: Palladin Institute of Biochemistry of NAS of Ukraine, No. 4, April 12, 2025.
Acknowledgement: We thank Cedars-Sinai Medical Center's International Research and Innovation in Medicine Program, and the Regional Cooperation for Health, Science and Technology Association (RECOOP HST Association) for their support.

Quantitative Detection of Early Soluble Fibrin in Blood Plasma Using I-5A Antibody

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Keywords: antibodies, thrombosis, plasma, fibrin, ELISA

Introduction: Soluble fibrin (SF) is generated during the early stages of thrombus formation and is recognized as a biomarker for thrombosis. In contrast to D-dimer, which reflects the degradation of cross-linked fibrin on the later stages of the process, SF appears in plasma much earlier. This makes it a useful indicator for detecting the initial phases of thrombogenesis. SF, circulating in the bloodstream for some time, can be partly hydrolysed by the fibrinolytic system. Therefore, the quantitative measurement of early SF versus total SF may serve as a valuable diagnostic tool for identifying the time of suffering from thrombophilia.
Aims: To develop a quantitative test system for detecting early forms of soluble fibrin using monoclonal antibody I-5A.
Methods: Monoclonal antibodies were produced in Palladin Institute of Biochemistry of NAS of Ukraine. Fibrin(ogen)-specific antibodies II-4d or I-5A were used as tag-antibodies, antibody I-3C was used as a catch-antibody. I-5A (specific towards Aα537-595) was used to measure early forms of SF, while II-4d (specific towards γ86-240) – for the total SF level. Optimal parameters for the new sandwich ELISA were established at 6 μg/mL for the I-3C capture antibody and 6 μg/mL for the biotinylated I-5A detection antibody. The developed ELISA system was tested on plasma samples from patients with burns (provided by Kyiv City Hospital #2) or barotrauma (Otolaryngology Institute of NAMS of Ukraine).
Results: The system was evaluated on plasma samples from patients with acute burn trauma (n=14), barotrauma (n=22) and healthy donors (n=12) as a reference group. In the burn trauma group, patients exhibited a pronounced prothrombotic state, confirmed by significantly elevated D-dimer (Median: 583 ng/mL vs. 80 ng/mL in reference group, $p<0.05$). This group also showed a statistically significant increase in total SF (Median: 18.3 μg/mL vs. 2.7 μg/mL, $p<0.05$). The I-5A monoclonal antibody assay revealed that the concentration of early SF forms increased by 20 % in these patients. In the barotrauma group, D-dimer was also elevated (Median: 150 ng/mL, $p<0.05$). However, unlike the burn group, these patients showed not statistically significant ($p > 0.05$) increase in either total SF or early SF forms detected by the I-5A assay in any patient.
Conclusion: Regarding the early SF levels in patient groups, we hypothesize that the difference may reflect the distinct origins and durations of the inflammatory processes in these pathologies. The developed ELISA system, based on monoclonal antibodies I-3C and I-5A, enables the sensitive and quantitative detection of SF and its early forms in blood plasma. This assay may serve as a practical tool for early thrombosis diagnosis and for monitoring coagulation activation in trauma-related pathologies.
Source of research support: Works were conducted within the framework of the ALLEA grant EFDS-FL2-02.
Ethical Committee Approval: Palladin Institute of Biochemistry of NAS of Ukraine (#20, 08/07/2025).
Acknowledgements: We thank Cedars-Sinai Medical Center's International Research and Innovation in Medicine Program, and the Regional Cooperation for Health, Science and Technology Association (RECOOP HST Association) for their support.

Altered Expression of miRNAs Involved in the Regulation of Antioxidant Enzymes' Expression in Patients with Hypertension and Chronic Kidney Disease

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Keywords: microRNAs, antioxidants, renal insufficiency

Introduction: Chronic kidney disease (CKD) is associated with inflammation and oxidative processes that have systemic consequences, such as cardiovascular disease. Previously, we demonstrated increased oxidative stress in CKD, with altered superoxide dismutase (SOD) activity. miRNAs are small non-coding RNAs which regulate post-transcriptional gene/protein expression. The present study hypothesized that there is a dysregulation of miRNAs' expression which regulate antioxidative enzymes SOD2/SOD3 and GPx4 expression, involved in control of oxidative stress.

Aims: This study aimed to evaluate miRNAs hsa-miR-21 (regulatory for SOD2/SOD3) and hsa-miR-532-3p (regulatory for GPx4) expression in patients with CKD and hypertension compared to healthy persons.

Methods: The study included 30 participants, divided into three groups: control group (N=10), participants with hypertension (N=10), and participants with CKD (stage 3-5, N=10). miRNA was isolated from peripheral blood mononuclear cells using the acid guanidine-thiocyanate and phenol-chloroform method. For detection and quantification, the commercial Applied Biosystems™ TaqMan™ Small RNA Assay kit was used. Relative expression of hsa-miR-21 and hsa-miR-532-3p was quantified by qRT-PCR (Bio-Rad, CFX96 Real – Time System) using hsa-miR-423-3p as an endogenous control. Data were normalized using the 2^(-ΔΔCt) method and expressed as fold change relative to control. The significance level was set at $p < 0.05$. Statistical analyses were performed by GraphPad Prism v6.01 and Microsoft Excel 2016.

Results: The 2^(-ΔΔCt) value of the target hsa-miR-21 was 1.26 (SD ± 0.83) in the control group, 20.94 (SD ± 13.51) in participants with hypertension and 11.49 (SD ± 6.01) in participants with CKD. The 2^(-ΔΔCt) value of the target hsa-miR-532-3p was 1.14 (SD ± 0.57) in the control group, 1.57 (SD ± 0.95) in participants with hypertension and 0.91(SD ± 0.43) in participants with CKD. The 2^(-ΔΔCt) value less than 1 indicates downregulation of miRNA expression. In this study, hsa-miR-21 expression was significantly upregulated in participants with hypertension and CKD compared to controls ($p < 0.0001$). Hsa-miR-532-3p expression showed a tendency toward upregulation in hypertension and downregulation in CKD compared to controls, however these differences were not statistically significant ($p = 0.1035$)

Conclusion: The results of this study indicate that hypertension and CKD are conditions with increased hsa-miR-21 (regulatory for SOD2/SOD3) which could lead to dysregulation of antioxidant defenses, a future venue of research. On the other hand, a large sample size could provide more definitive evidence for hsa-miR-532-3p.

Source(s) of research support: This study was supported by the Faculty of Medicine Osijek Institutional grant IP18-MEFOS-2024 (miRNA in mechanisms of vascular reactivity in humans, in animal models and cell cultures – 2024, Drenjančević I.), and IP20-MEFOS-2025 (miRNA in mechanisms of vascular reactivity in humans, in animal models and cell cultures – 2025, Drenjančević I.).

Ethical Committee Approval: The study was approved by the Ethics Committee of the University of J.J. Strossmayer in Osijek, Faculty of Medicine in Osijek CLASS: 641-01/24-01/04 REGISTRATION NUMBER: 2158-61-07-20-147 ISSUE DATE: 24.05.2024.

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Obtaining and Characterization of Proteinase with Fibrin(ogen)olytic Activity from the Culture Medium of *Bacillus sp.* L9

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Keywords: proteases, fibrin, defibrination, fibrin(ogen)olytic, Bacillus

Introduction: An increased tendency of blood to clot raises the risk of intravascular thrombus formation. One of the prospective therapeutic approaches to anticoagulation involves the use of fibrin(ogen)-specific proteases capable of regulating thrombus development. Fibrin(ogen)olytic enzymes of bacterial origin have gained growing interest due to their potential applications in medicine (particularly in thrombosis therapy) as well as in various biotechnological processes.
Aims: Therefore, the aim of this study was to isolate and purify the protease from the culture supernatant of *Bacillus* sp. L9 and to investigate its physicochemical properties as well as its specificity toward the fibrinogen molecule.
Methods: Fibrinogen was purified from human blood. Cells of the *Bacillus* sp. L9 were separated from the culture liquid medium by centrifugation. The supernatant was then subjected to ammonium sulfate precipitation, after which the resulting protein fraction was collected, dissolved in Tris-HCl buffer, and applied to a column packed with neutral TSK-Toyopearl HW-65F resin for further purification. Protein molecular weights were analyzed by SDS-PAGE, and enzyme electrophoresis was used to identify proteolytically active components. Fibrinogen hydrolysis products were examined by Western blotting using mouse monoclonal antibodies II-5C (anti-Aα20-78). The effects of a broad panel of cations on enzyme activity were evaluated, and inhibitor sensitivity was examined using EDTA, o-phenanthroline, DTT, L-cysteine, β-mercaptoethanol, PCMB, N-ethylmaleimide, EDC, and PMSF.
Results: The protease obtained from the culture medium of *Bacillus* sp. L9 has a molecular mass of approximately 40 kDa, the specific fibrinogenolytic and fibrinolytic activities were 483 and 383 U/mg protein, respectively. This enzyme was a calcium-dependent serine protease that exhibited fibrin(ogen)olytic activity, specifically cleaving the C-terminal portions of the Aα-chain of fibrinogen. The studied protease has multiple cleavage sites on the Aα-chain of fibrinogen, resulting in degradation products with molecular masses of approximately 50, 40, and 35 kDa.
Conclusion: Given its ability to efficiently cleave the fibrin(ogen) molecule, the enzyme may have potential applications in future antithrombotic therapy. By specifically degrading fibrinogen molecules and fibrin deposits in the bloodstream, this enzyme could reduce procoagulant activity and help prevent thrombotic complications.
Source of research support: NAS of Ukraine, № 0124U000251.
Ethical Committee Approval: Not applicable.
Acknowledgement: We thank Cedars-Sinai Medical Center's International Research and Innovation in Medicine Program, and the Regional Cooperation for Health, Science and Technology Association (RECOOP HST Association) for their support.

Properties of a New Haemostatic Agent Based on Modified Collagen Matrices

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Keywords: collagen, hemostatic, bleeding, enzyme, clotting

Introduction: Heavy bleeding poses a significant challenge in medicine, particularly during combat operations, accidents, and surgical procedures. The use of effective hemostatic agents can greatly reduce complications and mortality rates. As a natural biomaterial, collagen offers substantial potential for developing hemostatic agents due to its biocompatibility, biodegradability, and suitability for immobilizing active components.
Aims: This study aimed to develop modified collagen matrices incorporating an enzymatic coagulation activator and to assess their hemostatic efficacy.
Methods: Collagen was isolated from calfskin through acid hydrolysis, characterized via SDS-PAGE, and sterilized by autoclaving at 134 °C and 210 kPa for 35 minutes. Sterility was evaluated based on the absence of gram-negative and gram-positive microorganisms and yeasts. The enzymatic coagulation activator (EA) was purified from *Echis multisquamatis* venom using ion-exchange chromatography. Modified collagen matrices were prepared through lyophilization with the LyoQuest Telstar system. The activity of EA in combination with the collagen matrices and the quality of immobilization were assessed using the specific chromogenic substrate HD-Pro-Phe-Arg-pNA•2HCl. The local tolerance of the EA-modified collagen matrix was evaluated in Wistar Han rats through subcutaneous implantation, following the ISO 10993-6:2011 guidelines. Additionally, a liver injury model in rats was employed to assess the hemostatic efficacy of the modified collagen matrices.
Results: A collagen concentration of 300 mg/cm² and an EA concentration of 10 µg/cm² were selected to prepare collagen matrices. It was confirmed that EA maintained its enzymatic activity after lyophilization within the collagen matrix. In a buffer system, less than 10% of EA was released from the matrix, and no more than 5% of EA diffused out from the clot formed by the matrix in blood plasma. Collagen matrices measuring 3x3 cm and weighing 3 mg, modified with EA, were prepared. Their sterility was proven by microbiological study, and good local tolerance was confirmed through subcutaneous implantation tests. In a rat parenchymal liver bleeding model, the collagen matrices modified with EA achieved complete bleeding control within 15 seconds, compared to 60 seconds for a commercially available collagen-based sponge of the same weight.
Conclusion: The developed modified collagen matrices with EA are a safe and effective fast-acting hemostatic agent. They ensure rapid bleeding control and hold significant potential for further development and implementation in medical practice.

Source of research support: NAS of Ukraine, 0124U000251.
Ethical Committee Approval: Palladin Institute of Biochemistry of NAS of Ukraine #3, 05.03.2025.
Acknowledgement: We thank Cedars-Sinai Medical Center's International Research and Innovation in Medicine Program, and the Regional Cooperation for Health, Science and Technology Association (RECOOP HST Association) for their support.

The Splenectomy Influences the Outcomes of Ischemic Brain Lesion in the Mouse

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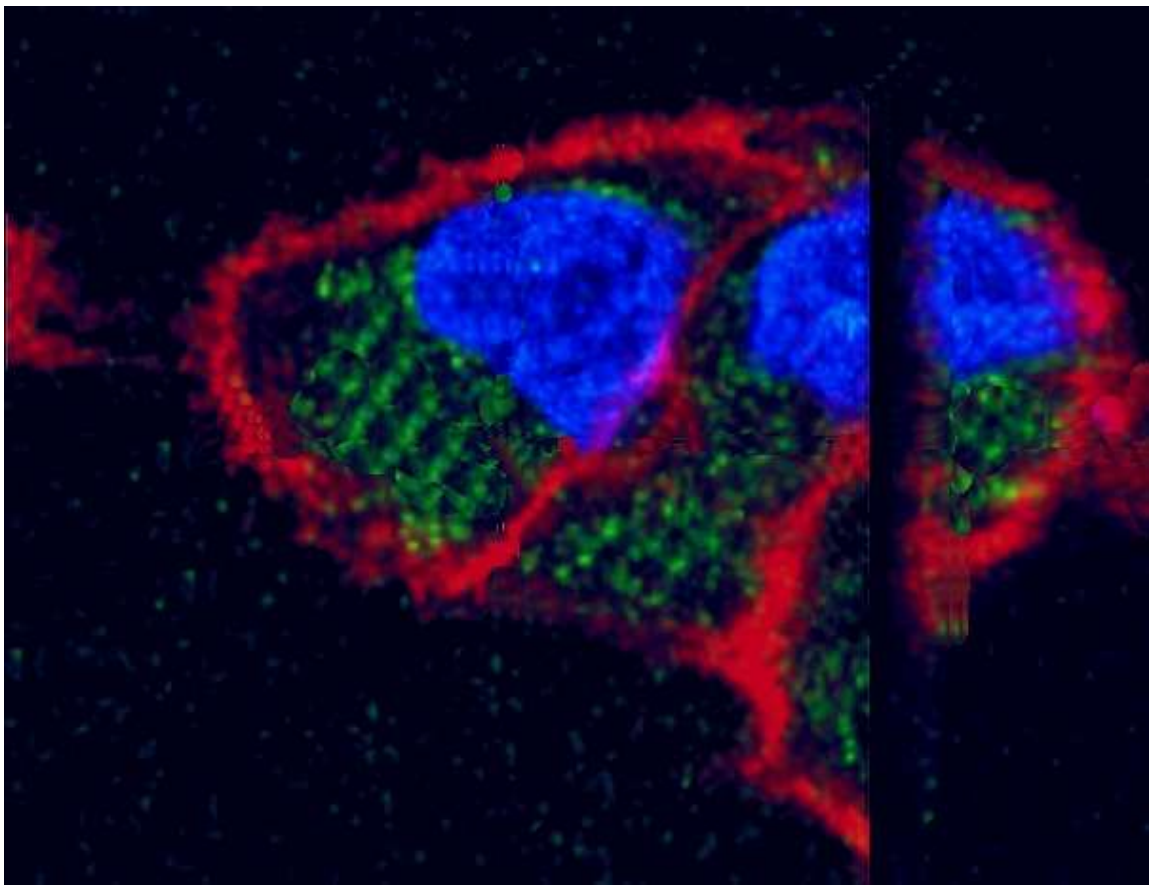
Keywords: spleen, ischemic stroke, inflammation, neural networks, monocytes

Introduction: Spleen appears to be important site of circulating macrophages, which could be mobilized according to the need. The brain damage, which results from the ischemic stroke, represents such an event, where the subsequent neuroinflammation, brain damage, and repair are mediated by cells residing in the brain (predominantly microglia and astrocytes), but as well the cells which arrive to the lesion site and contribute to these processes (e.g. macrophages).
Aims: To introduce and evaluate a preclinical intervention of splenectomy before the ischemic stroke
Methods: After splenectomy animals were allowed to recover, and subsequently ischemic brain lesion was induced by transient middle cerebral artery occlusion. The mice were longitudinally monitored afterwards for 28 days, structurally brain was assessed by in vivo MRI, in vivo bioluminescence of inflammation-related Tlr2, and functionally by neurological scoring. At the end of the study the brains were assessed by immunohistochemistry.
Results: The splenectomized animals after ischemic lesion had lower levels of bioluminescence related to neuroinflammation driven by Tlr2 promoter ($P = 0.032$) and better neurological outcome ($P = 0.044$) The analysis of the obtained results was complemented by innovative statistical modelling, logistic/multinomial regression classification, and neural networks application, which allowed to combine and compare multiple types of data belonging to single animal. Subsequently, the major effect was the change of the temporal dynamics of the post-stroke events. However, supporting the previous controversies, the decrease in neuroinflammation could not be declared as clearly beneficial nor detrimental.
Conclusion: The challenge of therapeutic interventions after ischemic stroke represented here by splenectomy was nicely shown in this study. The multiple intertwined post-stroke cellular and molecular events of brain damage and repair represent a complex target, and in this study the major effect was time-dependent modulation of post-stroke neuroimmune processes. Subsequently, the future interventions may need to target the timing and dynamics of immune responses rather than attempt a uniform suppression or enhancement of post-stroke events.

Source(s) of research support: This study was supported by the KK.01.1.1.07.0071 project "Synergy of molecular markers and multimodal in vivo imaging during preclinical assessment of the consequences of ischemic stroke - SineMozak"
Ethical Committee Approval: University of Zagreb, School of Medicine no.: 380-59-10106-20-111/73; 9.5.2020.
Acknowledgements: We thank Cedars-Sinai Medical Center's International Research and Innovation in Medicine Program, and the Regional Cooperation for Health, Science and Technology Association (RECOOP HST Association) for their support. MRI scans were performed at Laboratory for regenerative neuroscience – Glowlab, University of Zagreb School of Medicine (RRID:SCR_022701).

Medical Imaging
(MI)



Glioblastosaurus

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All-in-One Polymer-Engineered Lanthanide-Based Upconverting Nanoparticles for Bioimaging

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Keywords: bioimaging, nanoparticles, surface-engineering, polymer, lanthanide

Introduction: Effective theranostics for serious diseases requires multifunctional all-in-one nanolabels that are imaged simultaneously by multiple techniques. Here, bioconjugated upconverting nanoparticles (UCNPs) have many advantages, the major one is the absence of interfering optical background and low nonspecific binding. The applications also involve nanoprobes for regenerative medicine, drug delivery, photodynamic therapy and monitoring nano/microplastics in environment.
Aims: To design new ultrasmall PEGylated $NaYF_4$:Yb,Er nanoparticle-based imaging probe.
Methods: The UCNPs were synthesized by combined high-temperature coprecipitation and hydrothermal treatment. Protective coatings investigated were based on PEG- or ^{125}I-radiolabeled PEG-neridronate, poly(4-styrenesulfonic acid-*co*-maleic acid), copolymers of *N,N*-dimethylacrylamide, azidopentanoyl-RGDS-NH_2 and TAT-NH_2 peptides, or silica. Characterization methods involved transmission electron microscopy, dynamic light scattering, spectrofluorometry and several imaging modalities.
Results: UCNP size was tuned by varying reaction parameters. Depending on the selection of coating and composition of nanoparticles, the UCNPs were useful for *in vivo* bimodal SPECT/CT and optical tissue imaging, multimodal down- and upconversion luminescence, MRI, SPECT and X-ray CT bioimaging to non-invasively monitor biodistribution in organs, quantify pancreatic β-cell mass and determine Langerhans islet viability and functionality after transplantation.
Conclusion: Newly designed surface-engineered UCNPs are a promising imaging probe for the diagnosis of various diseases with high sensitivity and resolution.

Financial Support: The study was supported by the Czech Science Foundation (No. 25-16155S).
Acknowledgments: We thank Cedars-Sinai Medical Center's International Research and Innovation in Medicine Program, and the Regional Cooperation for Health, Science and Technology Association (RECOOP HST Association) for their support.

Long-term *in vivo* Evaluation of Ischemic Brain Damage Evolution in Bradykinin B2 Receptor–Deficient Mice

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Keywords: brain ischemia, bradykinin type 2 receptor, neuroprotection, glucose metabolism

Introduction: Bradykinin, a pro-inflammatory and vasoactive peptide released during brain ischemia, is known to increase vascular permeability and aggravate neurological injury. However, growing evidence suggests that activation of the bradykinin type 2 receptor (B2R) may exert neuroprotective effects, pointing to a potential dual role for this pathway in ischemic pathology.
Aims: In light of growing evidence linking B2R signaling to glucose metabolism and post-ischemic recovery, we carried out a longitudinal *in vivo* study in a mouse model of middle cerebral artery occlusion (MCAO) to investigate the role of B2R in ischemic progression and its potential contribution to metabolic regulation.
Methods: Intraluminal 30-minute Koizumi MCAO method was performed on four-month-old male C57Bl/6J and B2R-deficient (B2R-KO) mice to induce focal cerebral ischemia. Neurological scoring, behavioral testing, and magnetic resonance imaging (MRI) were conducted 7 days before surgery and 2, 9, and 35 days post-MCAO. Blood glucose levels were measured before and after ischemia. Image analysis was carried out using ImageJ.
Results: MRI analysis showed no significant differences in acute ischemic lesion volume between groups. However, by day 35 post-MCAO, B2R-KO mice displayed significant ipsilateral hemisphere atrophy, accompanied by pronounced neuronal loss, astrogliosis, and increased arterial volume relative to controls. B2R deficiency was further associated with more severe acute neurological impairments and compromised long-term functional recovery. Additionally, blood glucose levels were influenced both by the absence of B2R and by chronic post-ischemic changes.
Conclusion: Longitudinal multimodal analysis showed that B2R deficiency leads to worse neurological outcomes, delayed recovery, and progressive brain tissue loss, supporting a protective role of B2R during both acute and chronic ischemia. Additionally, altered glycemic profiles indicate a metabolic role for B2R and highlight the long-term impact of cerebral ischemia on glucose homeostasis.

Source(s) of research support: The study was funded by the Croatian Science Foundation project BRADISCHEMIA (UIP-2017-05-8082).
Ethical Committee Approval: University of Zagreb School of Medicine (380-59-10106-18-111/49, 25.02.2018).
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Real-Time Label-Free Phase Imaging with Digital Holographic Microscopy

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Keywords: microscopy, holographic, image processing, computer-assisted, optical phenomena

Introduction: Digital Holographic Microscopy (DHM) is a quantitative, label-free imaging technique that reconstructs the optical phase of transparent biological tissues. Due to its ability to reveal microstructural information without staining and to provide immediate visualization, DHM is well suited for supporting intraoperative assessment during brain tumor resections, offering surgeons rapid structural insight directly from fresh tissue.
Aims: This work presents the fundamental principles of DHM and the features that make it inherently compatible with intraoperative workflows in neurosurgery, highlighting the types of structural information that DHM can deliver when visualizing brain tissue.
Methods: DHM records interference patterns formed between a reference beam and light modified by the specimen. Numerical reconstruction yields quantitative phase images reflecting optical path length variations and exposing differences in cellular density, tissue organization, and microstructural irregularities. Because the technique operates on fresh, unstained samples and provides rapid reconstruction, it naturally fits the time-critical environment of surgical procedures. When combined with AI-based analytical tools, the quantitative nature of DHM images enables automated extraction of diagnostic features.
Results: DHM generates real-time phase images that differentiate healthy from infiltrated tissue through microscopic structural differences and variations in optical thickness. These objective, label-free measurements can serve as reliable input for AI algorithms, which may further support detection of tumor infiltration at the resection margin. In this way, DHM augmented by AI-driven analysis can provide immediate, structured feedback without disrupting surgical workflow.
Conclusion: Digital Holographic Microscopy is a fast, non-destructive, and operationally practical method for real-time structural assessment of brain tissue during tumor surgery. Its quantitative output makes it well suited for AI-supported interpretation, enhancing its potential to improve intraoperative decision-making and the precision of neurosurgical resections.

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Ethical Committee Approval: -
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Water-Dispersible Single-Particle Upconverting Nanothermometers Stabilized with Imidazolium-Based Ionic Liquids

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Keywords: single-particle nanothermometers, lanthanides, imidazolium-based ionic liquids

Introduction: The great demand for the development of highly-sensitive, precise and non-invasive nanothermometers arose over a decade ago, due to the rapid advancement of nanotechnology and biomedicine. So far, various luminescent nanothermometers have been developed; nevertheless, several challenges remain. Recently, lanthanide-doped upconverting nanoparticles have gained much attention, due to their biocompatibility and unique optical properties, such as temperature-sensitive and long-lifetime luminescence, sharp emission lines and negligible auto-fluorescence. Thus, they are highly promising for applications such as long-term biosensing, multimodal cell imaging and phototherapies of critical diseases.
Aims: The aim of this work was to synthesize highly monodisperse upconverting lanthanide-based nanoparticles and enhance their long-term stability in aqueous environment using imidazolium-based ionic liquids, enabling the development of nanothermometers for applications in nanomedicine.
Methods: The core and core-shell upconverting nanoparticles (UCNPs) were synthesized from anhydrous lanthanide chlorides (YCl_3, $YbCl_3$ and $ErCl_3$) and covered with oleic acid molecules. In the next step, the obtained UCNPs were transferred into the water solution and stabilized with a small amount of carboxy-functionalized imidazolium-based ionic liquids. All samples were analyzed using transmission electron microscope, atomic force microscope and two-color wide-field fluorescence microscope.
Results: The synthesized core and core-shell UCNPs were highly monodisperse, revealing round and rounded-rectangle shapes, respectively, as well as very strong luminescence in the temperature range of 25-50 °C. The addition of carboxy-functionalized ionic liquids to the aqueous dispersion of UCNPs significantly enhanced their stability and helped maintain high luminescence over time, even in water, which normally induces strong quenching.
Conclusion: We concluded that the applied carboxy-functionalized imidazolium-based ionic liquids can chemically and physically interact with the positively charged surface of UCNPs, hindering the access of water molecules to the nanoparticle surface. The resulting water-dispersible single-particle nanothermometers exhibited superb photostability, strong luminescence and an absence of photoblinking.
Source(s) of research support: We gratefully acknowledge the Polish National Science Center (grant SONATA 16, no. 2020/39/D/ST5/03359) for the financial support.
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Molecular Docking Dynamics in Nanopores Using Defocused Wide-Field Imaging

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Keywords: defocused wide-field imaging, single-molecule microscopy, docking molecules in nanopores

Introduction: The docking of biomolecules, including protein-DNA complexes, onto nanopores is a fundamental phenomenon that is employed in single-molecule sensing and DNA sequencing. Currently, the investigation of this process is solely conducted through the use of electrical methods, which involve monitoring ionic current time traces. While the efficacy of electrical measurements is well-documented, there are significant limitations with regard to resolving the intricate structural dynamics and specific spatial orientations of docked molecules. Consequently, the precise mechanism of docking molecules in nanopores remains insufficiently understood.
Aims: The primary aim of this study is to employ Defocused Wide-field Imaging (DWI) as a novel optical approach to visualize and characterize the dynamics of molecules docking in nanopores. The objective of this study is to determine the orientation and rotational behavior of individual molecules during the docking process, thereby overcoming the limitations of purely electrical detection.
Methods: We use a modified widefield fluorescence microscopy technique (DWI) where small, well-controlled defocus of the image is intentionally introduced. As a result, the typical dot-like fluorescent image of individual molecules expands into a complex pattern. These patterns carry information about the orientation of the molecule's transition dipole moment in and out of the image plane.
Results: The initial experimental proof-of-concept was successfully obtained by recording characteristic defocused emission patterns of labelled DNA molecules docked within the nanopore. The observation of these complex patterns confirms that the optical signal is strong enough to be detected in the experimental setup. Preliminary qualitative assessment indicates that the patterns contain angular information necessary for future detailed analysis of molecular orientation.
Conclusion: These initial results demonstrate the feasibility of combining DWI with nanopore technology. The ability to distinguish dipole-dependent patterns justifies the experimental approach. This optical method shows great promise for providing new mechanistic insights into the docking process.

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Ethical Committee Approval: - N/A
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Advancing Biological Image Quantification through Machine Learning Driven Segmentation

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Keywords: automated quantification, image analysis, machine learning, neuronal morphology

Introduction: Accurate quantification of complex biological structures is often hindered by their diverse shapes, sizes, and organizational levels, ranging from subcellular components to larger tissue architectures. Conventional FIJI (ImageJ) tools frequently face limitations in handling this variability, resulting in reduced accuracy and slower processing.
Aims: To develop a machine learning based approach capable of delivering fast, reliable, and high-precision morphometric analysis for both 2D and 3D biological imaging.
Methods: The workflow uses supervised machine learning for image segmentation, paired with adjustable intensity and size thresholds to refine object detection. Particle analysis quantifies area or volume, object number, and signal intensity, while skeletonization extracts morphometrics such as segment length, branching complexity, and width. The pipeline outputs 29 morphometric parameters suitable for a wide range of biological structures.
Results: Compared with commonly used open-source tools, the method achieved nearly threefold faster processing and markedly fewer false positives and negatives. Enhanced segmentation accuracy enabled the detection of fine structural features often missed by threshold-based approaches. The workflow performed consistently across diverse biological systems, including neuronal projections, mitochondrial networks, and vascular structures.
Conclusion: Combining machine learning segmentation with advanced morphometric and colocalization metrics provides a versatile, high-throughput solution for complex biological imaging. Improved accuracy, speed, and noise resilience make it suitable for demanding datasets in neuroscience, cell biology, and regenerative research. Its adaptability across multiple structural scales positions this method as a robust and broadly applicable analytical tool.

Source(s) of research support: This study was supported by projects of the Croatian Science Foundation entitled DEVDOWN (IP-2022-10-4656) and BRADISCHEMIA (UIP-2017-05-8082).
Ethical Committee Approval: University of Zagreb School of Medicine (380-59-10106-18-111/49, 25.02.2018).
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Early Sonographic Triad Leading to First-Trimester Diagnosis of Meckel–Gruber Syndrome – Case Report

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Keywords: prenatal screening, deep phenotyping, nuchal translucency, NIPT, amniocentesis

Introduction: Meckel–Gruber syndrome (MGS) is a rare, lethal pathology characterized by the classic triad of occipital encephalocele, cystic renal dysplasia, and postaxial polydactyly. Although diagnosis is typically made in the late first or second trimester, high-resolution ultrasound and early anatomic assessment allow earlier diagnosis. Early identification is essential for counseling and pregnancy management.
Aims: The aim of this paper is to report a case of Meckel–Gruber syndrome diagnosed at 13 weeks of gestation during a routine scan.
Methods: A 20-year-old gravida 3, para 2 woman known with a 12 weeks pregnancy presented for vaginal bleeding. Transabdominal and transvaginal ultrasound examinations were performed where polycystic kidneys were observed. Follow-up evaluation included targeted neurosonography and skeletal assessment. Genetic analysis recommendations included targeted ciliopathy gene sequencing.
Results: Ultrasound revealed a large occipital encephalocele, markedly enlarged echogenic kidneys (both above the 95th centile for gestational age), and bilateral postaxial polydactyly of the upper and lower limbs. Amniotic fluid volume was normal.
The patient opted for termination of pregnancy the next day after diagnosis. The sonographic consistent with Meckel–Gruber syndrome. Post-termination examination verified the prenatal findings.
Conclusion: This case demonstrates that Meckel–Gruber syndrome can be reliably identified in the early first trimester using detailed ultrasound. Recognition of characteristic early features – particularly encephalocele and renal enlargement – supports timely counseling and management in affected pregnancies.

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Acknowledgements: We thank Cedars-Sinai Medical Center's International Research and Innovation in Medicine Program, and the Regional Cooperation for Health, Science and Technology Association (RECOOP HST Association) for their support.

Fetal Speckle Tracking Echocardiography in Pregnancies with Intrauterine Growth Restriction

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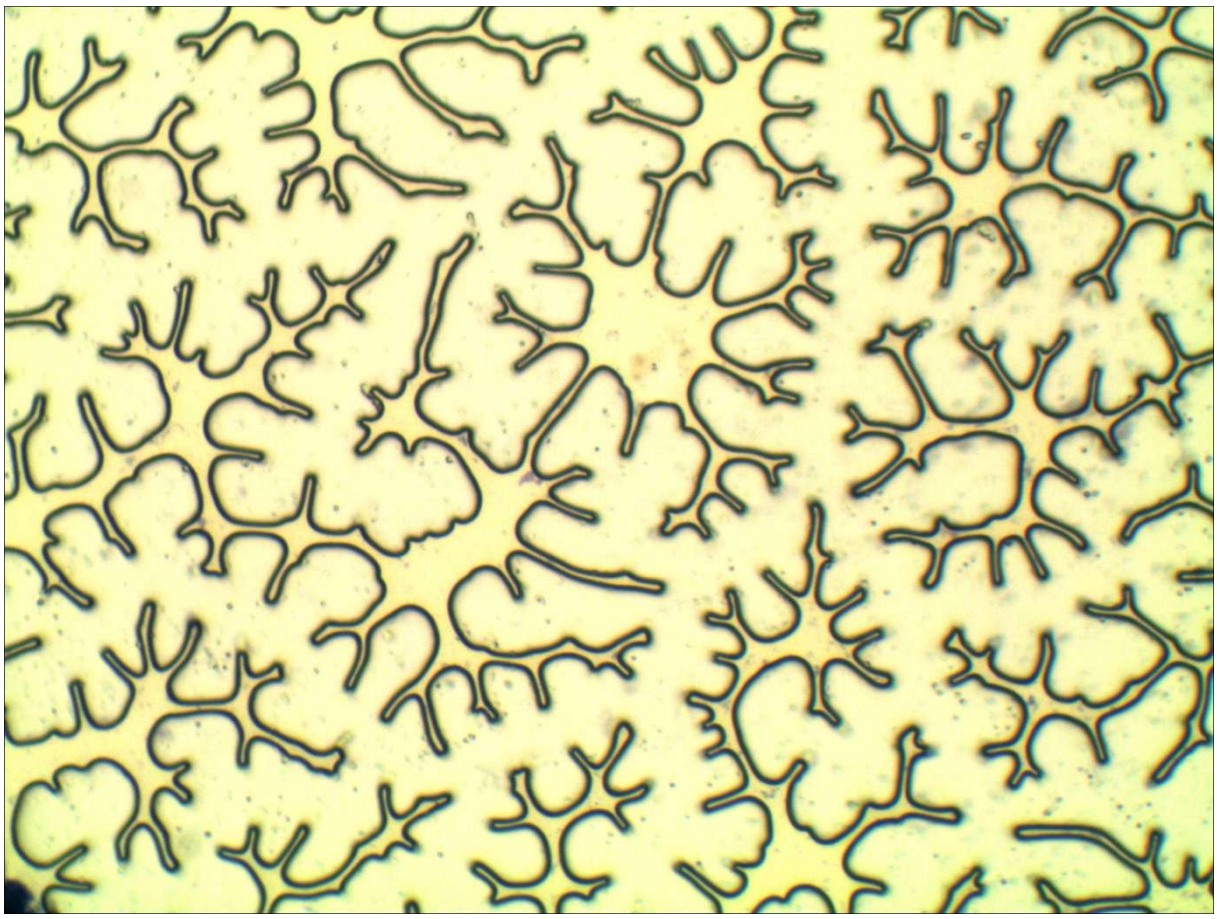
Keywords: IUGR, speckle tracking, prenatal echocardiography, ultrasound markers

Introduction: Intrauterine growth restriction (IUGR) remains a significant global concern with lasting effects beyond pregnancy, requiring comprehensive fetal assessment through Doppler ultrasonography, including umbilical artery, middle cerebral artery (MCA), and ductus venosus (DV) evaluations, which help identify fetal compromise and guide delivery timing. Advances in cardiac assessment, particularly speckle tracking echocardiography (STE), provide sensitive evaluation of fetal myocardial function.
Aims: The aim of this paper is to present the experience of our clinic regarding the management of intrauterine growth restriction adding to the know sonographic markers, fetal echocardiography with speckle tracking.
Methods: This paper presents a single-center experience in prenatal evaluation of fetuses with intrauterine growth restriction and small for gestational age, compared with fetuses with normal development. The systems used to do the acquisition were Voluson Expert 22 (BT24) and a Voluson E8 (BT17) ultrasound system equipped with an RM7-C transducer and RAB-6. There were used the B-Mode of the apical or basal four-chamber view with video acquisition. All equipment used was manufactured by GE HealthCare (Europe). The analysis includes a number of 15 cases that were examined from the second trimester until delivery. The collected data included estimated fetal growth, cerebro-placentar ratio, left ventricle global longitudinal strain, ejection fraction, fractional area and cardiotocography patterns.
Results: Results have shown abnormal strain parameters, represented by global longitudinal strain, are modified in severe cases of intrauterine growth restriction and can be associated with adverse perinatal outcomes. The results are correlated with the compensation mechanism in the third trimester of pregnancy. The main limitation of this study is the small sample of patients.
Conclusion: Fetal cardiac evaluation has progressed through speckle tracking echocardiography, which provides a sensitive assessment of myocardial deformation and can reveal early cardiac dysfunction. Even if its accuracy remains limited by technical**,** speckle tracking echocardiography has deepened our understanding of myocardial performance in IUGR fetuses. In selected cases, integrating conventional Doppler parameters with advanced measures may help identify early cardiac impairment, distinguish true fetal growth restriction (FGR) from small-for-gestational-age (SGA) fetuses, and improve risk stratification.
Source of research support: no financial support.
Ethical Committee Approval: Ethical Committee of the "Dr. I Cantacuzino" Clinical Hospital. Bucharest. Decision no. 17573 from 28.08.2024.
Acknowledgements: We thank Cedars-Sinai Medical Center's International Research and Innovation in Medicine Program, and the Regional Cooperation for Health, Science and Technology Association (RECOOP HST Association) for their support.

Translational Medical Research (TMR)



Morphology of Solidified Polymer Adhesive on the Surface of a Histological Section Randomness That Became Structure

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Bradykinin Type 2 Receptor Deficiency Reshapes Acute Neuroinflammation and Improves Cell Survival after Ischemic Stroke in Diabetic Mice

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Keywords: diabetes mellitus, stroke, brain, microglia, bradykinin type 2 receptor

Introduction: Diabetes mellitus exacerbates cerebral ischemic damage by potentiating neuroinflammation. Previous studies suggest that activation of the bradykinin type 2 receptor (B2R), a mediator of inflammation and vascular dynamics, may be detrimental to the development of ischemic injury in diabetic animals.
Aims: To determine the effects of B2R deficiency on microglial response, neutrophil infiltration, and neuronal survival during the acute phase of cerebral ischemic injury development in a mouse model of type 1 diabetes.
Methods: Ischemic injury was induced in four-month-old male diabetic C57BL/6-Ins2^{Akita}/J (Akita, n=14) and diabetic B2R knockout B6.Cg-Ins2^{Akita}/Bdkrb2^{tm1Jfh}/SmiJ (Akita/B2R-KO, n=15) mice by a 30-minute middle cerebral artery occlusion (MCAO) and confirmed by magnetic resonance imaging. On the first and third day following MCAO, a comprehensive immuno-histochemical analysis was performed to quantify changes in microglial, neutrophil, and neuronal populations.
Results: Our findings revealed similar ischemic lesion volumes in both groups. However, the extensive loss of microglial cells, evident in both hemispheres, was significantly delayed by B2R deficiency ($p<0.0001$). Morphological analysis of the remaining microglial cells showed a decrease in the ramification index within the lesion and, to a lesser extent, in the contralateral hemisphere of Akita mice, reflecting a higher percentage of ameboid cells within the population. In Akita/B2R-KO mice, microglial reactivity to ischemia and the associated morphological change were significantly reduced, particularly in the contralateral hemisphere ($p<0.0001$). The attenuated immune response in diabetic B2R-deficient mice was accompanied by a marked decrease in neutrophil infiltration within the ischemic territory (cortex $p<0.5$, striatum $p<0.0001$) and improved neuronal survival in both hemispheres ($p<0.0001$) compared to Akita controls.
Conclusion: This study uncovers a novel role of B2R in microglia-neutrophil-neurons interactions after cerebral ischemia. These findings suggest that targeting the B2R signaling pathway may have significant therapeutic potential for mitigating acute neuroinflammation after ischemic stroke in diabetic patients.
Source of research support: The study was supported by the Croatian Science Foundation project BRADISCHEMIA (UIP-2017-05-8082).
Ethical Committee Approval: University of Zagreb School of Medicine (380-59-10106-18-111/49, 25.02.2018).
Acknowledgements: We thank Cedars-Sinai Medical Center's International Research and Innovation in Medicine Program, and the Regional Cooperation for Health, Science and Technology Association (RECOOP HST Association) for their support.

Sex Differences in Oxidative Stress and Myocardial Morphology in a Rat Model of Post-Traumatic Stress Disorder

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Keywords: Post-Traumatic Stress Disorders (PTSD), oxidative stress, myocardium, sex, rats

Introduction: The ongoing war and prolonged psychological stress experienced by Ukrainian citizens have resulted in a notable increase in cases of Post-Traumatic Stress Disorder (PTSD). This condition is linked to a heightened risk of cardiovascular diseases. Although epidemiological data suggest that women are more vulnerable to PTSD, the specific characteristics of cardiac injury related to sex remain insufficiently explored.
Aims: to study the impact of PTSD on the heart in rats of different sexes.
Methods: PTSD was modeled using the Single Prolonged Stress (SPS) paradigm, a preclinical model that recapitulates the behavioral, molecular, and physiological alterations observed in PTSD. 48 Wistar rats (mixed sex, 180-200 g, 4 months old) were divided into four groups: Control Males (I), Control Females (II), PTSD Males (III), and PTSD Females (IV). The SPS procedure consisted of 120 minutes of restraint, followed by 20 minutes of forced swimming, and subsequent ether exposure until general anesthesia was induced. Blood serum (diene conjugates, DK, thiobarbituric acid reactive substances TBRs, oxidative modification of proteins, OMP, superoxide dismutase (SOD) and catalase activity) and heart tissue were analyzed.
Results: PTSD activated lipid peroxidation processes were significantly higher in females. While OMP was more dominant in males. In rats of III and IV groups SOD activity decreased in both sexes (more in females), a sharp increase in catalase activity was observed. We may suggest intense mobilization of antioxidant defenses in response to oxidative stress. Morphological analysis revealed structural deviations in the hearts of PTSD rats compared to intact controls. Key findings included marked cardiomyocyte edema, vascular congestion, and hemodynamic disorders. Microscopic examination revealed moderate lymphohistiocytic infiltration, separation of individual muscle fibers, and destruction of cardiomyocytes accompanied by pronounced stromal edema.
Conclusions: PTSD leads to activation of oxidative stress and structural myocardial changes characterized by distinct sexual dimorphism. Female sex may be associated with more severe structural cardiac injuries in PTSD due to the exhaustion of cell membrane adaptive reserves.
Source(s) of research support: Research was supported by TNMU.
Ethical Committee Approval: The study was approved by the Bioethics Committee of the I. Horbachevsky Ternopil National Medical University, Protocol No 83 from November 3, 2025.
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Validation of a New Automated Method for Determining Overall Hemostatic Potential

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Keywords: hemostasis, overall hemostatic potential, diabetes

Introduction: The method for measuring the overall hemostatic potential (OHP) is based on calculating the parameters of the turbidimetric curve of blood plasma clot formation and destruction. It provides information about an individual patient's blood plasma's ability to form a clot, hydrolyze it, and maintain hemostatic balance. Detection of changes in the curve during clot formation and hydrolysis is performed using an automatic measurement system in microplates. Performing the test requires specialized equipment, and the process of analyzing the results is not automated, requiring a highly qualified researcher. Compacting and automating the process of determining the OHP can expand the possibilities of using the method and ensure its implementation.

Aim: Construction and validation of the portable device "Hematotester" for measuring the OHP of blood plasma.

Methods: Plasma samples from healthy donors (n = 7) and patients with diabetes (n = 5) have been tested using the "Hematotester" and a reader (Thermo Multiscan). OHP was characterized by the area under the turbidity curve of the clot from the moment of initiation of plasma coagulation using ATTP-reagent to the moment of complete destruction of the clot in the presence of t-PA. Statistical analysis was performed using the Deming regression, Mann-Whitney and Bland-Altman tests.

Results: One parameter of the OHP –the maximum rate of growth of the clot turbidity (V1; $p = 0.00578$) – was significantly higher in the diabetes group. The magnitude of the final turbidity of the clot (H; $p = 0.05118$) has been close to be a significantly different. To compare the obtained results the Deming regression (to estimate the linear relationship) and the Bland-Altman method (to assess the agreement between two different methods) were used. Deming regression showed a correlation between the methods (Pearson coefficient 0.945). However, the regression analysis and Bland-Altman plot demonstrated a significant systematic bias (error), which should be eliminated in further validation of the instrument.

Conclusions: The possibility of using the Hematotester device for rapid measurement of the OHP of blood plasma has been demonstrated. A regression analysis confirmed the correlation of the values obtained using the "Hematotester" and using the reader. The parameters measured by the device demonstrate pathological shifts in the blood coagulation system during disease; however, further use of the device requires refinement. Additional studies involving a larger number of samples are needed.

Sources. Scientific research project: ”Study of non-enzymatic activation of prothrombin“. State registration number: 0124U002143.

Ethical approval. The human study protocol was approved by the Committee on Biosafety and Bioethics of the Palladin Institute of biochemistry NAS of Ukraine (protocol №8, 29.10.2024).

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The Effect of Frozen and Lyophilized Human Cord Blood Serum on L929 cells

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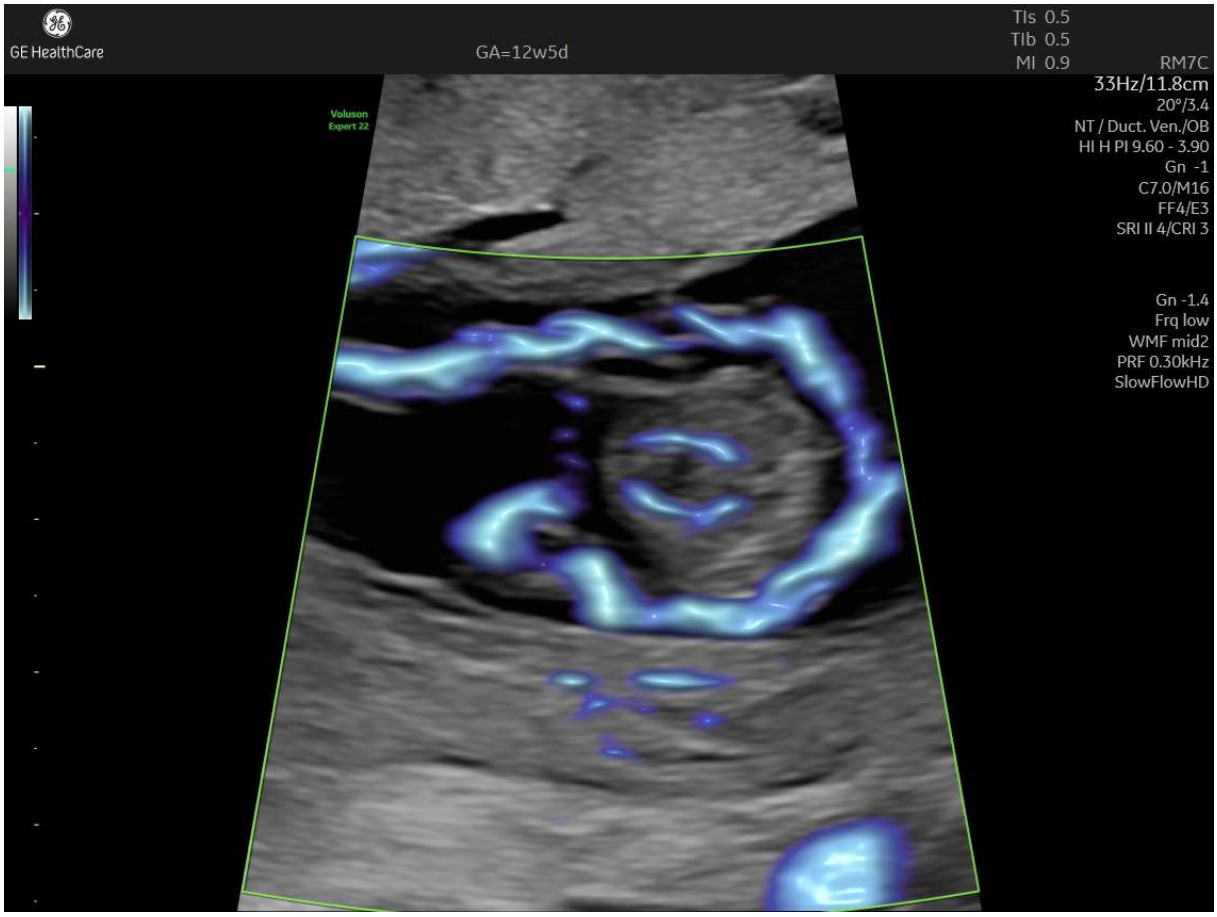
Keywords: lyophilization, freezing, cord blood serum, L929, regenerative medicine

Introduction: Research into the mechanisms of human cord blood components action and development allows for a more in-depth assessment of the therapeutic potential of biologically active compounds contained in cord blood serum. Cord blood serum is an underrated biostimulant that can be used in the treatment due to the large number of growth factors and biostimulants it contains. The necessary conditions for the use of cord blood serum are the creation of storage technologies, which is possible with the help of deep freezing or lyophilization.
Aims: Compare and analyze changes in the life processes of L929 cells after adding different concentrations of frozen and lyophilized human cord blood serum.
Methods: We analyzed cord blood serum frozen at –20 °C, as well as samples lyophilized under two different pre-cooling conditions (–20 °C and –80 °C). Cord blood serum was added to L929 cells at 1%, 5%, and 10% relative to the volume of the cell culture dish. Changes in cellular metabolism were examined using the MTT assay, neutral red recovery assay, scratch assay, population doubling assay, and adhesion assay.
Results: The addition of serums to L929 cells at concentrations of 1% partially restored adhesion, but the least effect was observed with lyophilisate after prior cooling to –20 °C. At concentrations of 5% and 10%, all biostimulants provided adhesion and morphology similar to FBS. The time to double the population increased significantly without serums. The addition of frozen and lyophilized serum pre-cooled to –80 °C in all concentrations, as well as lyophilized serum pre-cooled to –20 °C in concentrations of 5 and 10%, significantly accelerated proliferation; the maximum effect was observed at 10%. Cell migration activity increased in direct proportion to the use of biostimulants and was highest at 10% frozen and lyophilized serum pre-cooled to –80 °C. According to the MTT test, all serums also directly increased the metabolic activity of mitochondria, while no effect on lysosomal activity was detected in the neutral red uptake test.
Conclusion: Frozen and lyophilized cord blood serum under various conditions stimulates L929 cell adhesion, proliferation, and metabolic activity in vitro and has minimal effect on lysosomal function. Lyophilization with prior cooling to –20 °C is accompanied by a decrease in biological activity of human cord blood serum compared to freezing and lyophilization after cooling to –80 °C.
Source of research support: SRW 2.2.6.145 (0121U113329) «Determining the role of pre-treatment in improving the effectiveness of cryopreservation and hypothermic storage of cell structures at various levels of organization».
Acknowledgments: We thank Cedars-Sinai Medical Center's International Research and Innovation in Medicine Program, and the Regional Cooperation for Health, Science and Technology Association (RECOOP HST Association) for their support.

Reproductive Health
(RPH)



Soft Flow Vision: Illuminating Umbilical Vascular Pathways

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Assessing Corticosteroid Effects on Fetal Lung Development Using Shear Wave Elastography in Preterm Birth Risk

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Keywords: fetal lung maturation, shear wave elastography, betamethasone, preterm birth

Introduction: Two-dimensional ultrasound shear wave elastography (SWE) has emerged as a promising tool for assessing fetal lung development and predicting neonatal respiratory outcomes. This study aimed to evaluate changes in fetal lung elasticity following betamethasone administration.

Aims: Assessment of fetal lung maturation using SWE during antenatal corticosteroid therapy in pregnancies with increased risk for preterm delivery.

Methods: A prospective observational study was conducted involving 34 pregnant women hospitalized for threatened preterm labor. Eight participants received direct fetal intramuscular betamethasone injections, whereas 26 received maternal intramuscular administration. SWE measurements of fetal lung and liver elasticity were performed immediately prior to corticosteroid administration, then repeated 1 minute and 24 hours after treatment.

Results: Direct fetal betamethasone injection did not produce an immediate change in lung elasticity, with measurements taken 1 minute post-injection showing no significant difference from baseline values. By contrast, a significant reduction in lung elasticity was observed 24 hours after treatment, decreasing from a range of [1.91–2.26] to [1.71–2.07]. Maternal betamethasone administration demonstrated a similar pattern: fetal lung elasticity remained stable immediately after injection but showed a significant decline 24 hours later, from [1.59–2.11] to [1.51–1.75]. Throughout the third trimester, lung elasticity decreased by an average of 12.4% following corticosteroid exposure, whereas fetal liver elasticity remained stable across all measurement time points. A physiologic, gestational age-related decrease in lung elasticity was also identified, with values declining significantly between early (24–27 weeks: [1.64–2.29]) and later (32–34 weeks: [1.52–1.87]) gestational periods.

Conclusion: Both direct fetal and maternal betamethasone administration resulted in a measurable decrease in fetal lung elasticity 24 hours after treatment, consistent with an acceleration of lung maturation to patterns typically seen at more advanced gestational ages. These findings underscore the potential clinical value of fetal lung elastography in guiding the management and counseling of high-risk pregnancies at risk of preterm birth.

Sources of support: Project no. RRF-2.3.1-21-2022-00012, titled National Laboratory on Human Reproduction has been implemented with the support provided by the Recovery and Resilience Facility of the European Union within the framework of Programme Széchenyi Plan Plus. This project has received funding from the HUN-REN Hungarian Research Network.

Ethical Committee Approval: The study was approved by the Regional and Local Research Ethics Committee of the University of Pécs, Hungary (approval numbers: PTE KK 7072-2018 and PTE KK 10086-PTE2025).

Acknowledgements: We thank Cedars-Sinai Medical Center's International Research and Innovation in Medicine Program, and the Regional Cooperation for Health, Science and Technology Association (RECOOP HST Association) for their support. Project no. RRF-2.3.1-21-2022-00012, titled National Laboratory on Human Reproduction has been implemented with the support provided by the Recovery and Resilience Facility of the European Union within the framework of Programme Széchenyi Plan Plus. This project has received funding from the HUN-REN Hungarian Research Network.

Investigating the Functions of Aquaporins in Regulating Uterine Contractions and Preventing Preterm Birth

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Keywords: AQP, uterus, pregnancy, preterm birth

Introduction: Aquaporin (AQP) channels are small integrated membrane proteins that are mostly permeable to water. Among others, they are expressed in female reproductive tissues and play an important role during pregnancy. Our research group previously found that the AQP5 isoform was dominant in the uteri of late-gestation rats, with its expression declining dramatically by the day of delivery.
The aim of our studies is to demonstrate the importance of AQP5 in childbirth and to investigate its mechanism in rat and human samples.
Methods: Our animal experiments were performed with pregnant Sprague-Dawley rats, while tissue and blood samples from different weeks of pregnancy were collected from the Albert Szent-Györgyi Medical Center Obstetrics and Gynecology Clinic for our human studies. The animals were treated with citral and AQP5 siRNA, and then the uterine tissue was removed for further examination. The *in vitro* contraction tests were performed in an isolated organ bath. In our molecular studies, AQP5 mRNA and protein expression were determined in tissue samples using RT-PCR and Western blot, respectively, while AQP5 concentrations in blood samples were measured using an ELISA kit.
Results: Citral treatment resulted in a dose-dependent decrease in the *in vitro* uterine contractions, a significant increase in AQP5 expression, and a significant delay in parturition in treated animals compared to control animals. After AQP5 siRNA treatment, *in vitro* uterine contractions increased, AQP5 expression decreased, and treated animals gave birth earlier than animals in the control group. In the human uterus, we measured significantly higher AQP5 expression at 34 weeks of pregnancy than in later weeks, and plasma AQP5 concentration was also significantly higher than in the last weeks and in the control group.
Discussion and Conclusion: Based on our results, we conclude that there is a strong correlation between AQP5 expression and uterine contractions. Preterm birth is a significant problem worldwide today, and the only way to address it is to understand the exact mechanisms of birth, to which our research has contributed and which we hope may form the basis for a new tocolytic target in the future.

Sources of financial support: This work was supported by the National Research, Development and Innovation Office, Hungary (grant NKFI-FK19-132499) and ”Geza Hetenyi“ Grant, University of Szeged [No.:5S 724 (A202)].
Animal Care and Use Committee Approval: The animals were treated in accordance with the European Communities Council Directives (2010/63/EU) and the Hungarian Act for the Protection of Animals in Research (Article 32 of Act XXVIII). All experiments involving animal subjects were carried out with the approval of the Hungarian Ethical Committee for Animal Research (registration number: IV/2767/2020**).**
Ethical Committee Approval: Human studies were conducted with the permission of the Regional Medical Research Council of the University of Szeged (Permission No. 52/2020-SZTE).
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The Non-Genomic Actions of 5α- and 5β-dihydrotestosterone on a Rat Uterus Model: *in vitro* and *in vivo* Study

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Keywords: dihydrotestosterone, flutamide, non-genomic, uterine contractility, pregnancy

Introduction: Premature labor and other conditions associated with uterine contractility represent important concerns in clinical practice and are associated with many complications and death among newborns. Androgens play important functions in the body via the interaction with the nuclear as well as non-nuclear receptors through different targets named "non-genomic actions. In previous studies, we confirmed the non-genomic uterus relaxing effect of testosterone (T) *in vitro* and *in vivo*.

Aims: Our research aimed to investigate the inhibitory effect of 5α- and 5β-dihydrotestosterone (DHT) on uterine contractility in non-pregnant and 22-day pregnant rats *in vitro* and *in vivo*.

Methods: In our *in vitro* experiments, preparations of non-pregnant and pregnant rat uterus were examined in an isolated organ bath. KCl-induced (25 mM) rhythmic contractions were measured in the presence of 5α- or 5β-DHT, with a cumulative dose (10^{-9}-10^{-3} M), at 5-minute time intervals. During *in vivo* experiments, a strain gauge is placed on the uterus of non-pregnant and pregnant Sprague-Dawley rats under ketamine-xylazine anesthesia. Contractions were recorded for 15 minutes after a single intraperitoneal dose of 3-300 mg/kg 5α- or 5β-DHT. The statistical analysis of the results was carried out with a paired t-test or an ANOVA test.

Results: For the *in vitro* experiment, 5α- and 5β-DHT exerted an inhibitory effect with 62-63% on non-pregnant and 52-69% on the pregnant uterine muscles, which was not affected by the presence of flutamide and bicalutamide (androgenic antagonists), mifepristone (progesterone and glucocorticoid antagonist), G15 (G protein-coupled estrogen receptor blocker), paxilline (K-channel blocker), callindol (calcium-sensing receptor activator), NPS (calcium-sensing receptor blocker), or the removal of the endometrium. They initiated a similar inhibitory effect of nifedipine (calcium channel blocker) on a cumulative potassium chloride and calcium chloride response. Furthermore, 5α- and 5β-DHT inhibited *in vivo* uterine contractions by 52% and 62% in non-pregnant rats, respectively. Similarly, they produced 46% and 58% relaxation in late pregnant rats in a flutamide-resistant manner, respectively.

Conclusion: 5α- and 5β-DHT produce rapid dose-dependent *in vitro* uterine relaxation via a non-genomic pathway, in which a calcium blocking effect similar to nifedipine may play a role. They also produce a rapid non-genomic flutamide-resistant *in vivo* uterine relaxation in a dose-dependent manner in the tested dose range. These relaxant effects might be useful clinically for the management of preterm labor, dysmenorrhea and endometriosis associated pain.

Ethical Committee Approval: All experiments involving animal subjects were carried out with the approval of the National Scientific Ethical Committee on Animal Experimentation (registration number: XIII./72/2020).

Acknowledgements: We thank Cedars-Sinai Medical Center's International Research and Innovation in Medicine Program, the Regional Cooperation for Health, Science and Technology Association (RECOOP HST Association) for their support of our study, and our organization as a participating Cedars-Sinai Medical Center – RECOOP Research Center.

Impact of Pasteurization on miRNA Composition in Human Breast Milk

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Keywords: breast milk, pasteurization, miRNA

Introduction: Human breast milk contains a diverse repertoire of microRNAs (miRNAs) that are thought to contribute to immune education and developmental programming in the infant. Despite widespread use of donor milk, the molecular consequences of thermal processing remain poorly understood.
Aims: The aim of the study was to investigate how pasteurization affects the microRNA composition of human breast milk using next-generation sequencing.
Methods: Total nucleic acids were extracted from pasteurized and non-pasteurized human breast milk samples and subjected to total RNA sequencing using a high-throughput next-generation sequencing platform. Bioinformatic analysis was used to quantify and compare miRNA abundance across groups.
Results: Clear separation of pasteurized and non-pasteurized samples was observed based on miRNA expression profiles. Pasteurization resulted in a pronounced downregulation of several miRNAs, including *hsa-miR-574-3p*, *miR-130b-3p*, *miR-193a-5p*, *miR-877-5p*, and *let-7b-3p* (adjusted $P < 1\times10^{-6}$), many of which are associated with immune regulation, epithelial integrity, and developmental signaling. A smaller subset of miRNAs, such as *miR-200b-5p* and *miR-146b-5p*, showed relative enrichment post-pasteurization, possibly due to structural stability or vesicle-mediated protection. Overall, pasteurization markedly altered the miRNA cargo of breast milk.
Conclusion: Next-generation sequencing of total nucleic acid extracts revealed significant loss of functionally relevant miRNAs following pasteurization. This shift in molecular composition may affect the biological efficacy of donor milk, particularly in premature or immunocompromised neonates.
Source of research support: National Laboratory for Human Reproduction University of Pécs, Medical School, Pécs, Hungary
Acknowledgement: We thank Cedars-Sinai Medical Center's International Research and Innovation in Medicine Program and the Regional Cooperation for Health, Science and Technology Association (RECOOP HST Association) for their support of our study and our organization as a participating Cedars-Sinai Medical Center – RECOOP Research Center (CRRC).

The Effect of Artificial Reproductive Techniques on the Hormonal Status of Newborns

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Keywords: *in vitro* fertilization, ovarian stimulation, metabolic status

Introduction: More than 10 million children have now been born worldwide through assisted reproductive technologies (ART). In Hungary, following international trends, the number of children conceived by ART has been steadily increasing for years.
Aims: In our study, we were determined to find out, whether the hormonal treatment used for ovarian stimulation influences the neonatal hormonal profile shortly after birth.
Methods: We analyzed data from 128 newborns, comparing 61 term infants conceived via *in vitro* fertilization (IVF) to 67 naturally conceived control infants. Blood samples were collected 24-72 hours after birth, and additional measurements, such as birth weight, gestational age and Apgar scores were taken into consideration. The following hormones were measured: TSH, total thyroxine, LH, FSH, estrogen, progesterone, total testosterone, cortisol, prolactin and insulin.
Results: There were no significant differences between the two groups in birth weight (3108 ± 539 g vs. 3358 ± 573 g), gestational age (38,7 ± 1,8 weeks vs. 38,4 ± 1,5 weeks) or Apgar scores (1-minute: 8,86 ± 0,39 vs. 8,97 ± 0,41). A male-to-female ratio was 0,91 in the IVF group and 1,18 in the control group. Infants conceived via IVF had significantly lower TSH (7,55 ± 0,52 vs 10,02 ± 1,11 mU/L), and higher plasma thyroxine concentrations (32,17 ± 0,59 vs. 29,73 ± 0,76 pmol/L). Oestradiol levels were also significantly higher in the IVF group compared to controls (128,00 ± 11,26 vs 96,74 ± 8,04 pmol/L). Male newborns conceived by IVF exhibited significantly higher plasma testosterone levels (5,77 ± 0,41 nmol/L) compared with both IVF-conceived females (3,81 ± 0,21 nmol/L) and naturally conceived females (4,3±0,39 nmol/L respectively). No significant differences were found in the remaining hormone parameters.
Conclusion: Our findings indicate that maternal hormonal stimulation influences neonatal hormonal status. In assisted reproduction, the aim is always to achieve individualized ovarian stimulation. Further studies are needed to determine the extent to which different stimulation protocols contribute to the hormonal differences observed in newborns.
Source of research support: Project no. RBF-2.3.1-21.2022.00012, titled National Laboratory on Human Reproduction has been implemented with the support provided by the Recovery and Resilience Facility of the European Union within the framework of Programme Széchenyi Plan Plus. This Project has received funding from the HUN-REN Hungarian Research Network.
Ethical Committee Approval: 8753-PTE 2021 of the Regional Research Ethics Committee of the University of Pécs
Acknowledgements: We thank Cedars-Sinai Medical Center's International Research and Innovation in Medicine Program, and the Regional Cooperation for Health, Science and Technology Association (RECOOP HST Association) for their support.

Impact of Metabolic and Hypertensive Pregnancy Conditions on Placental ABC Transporters

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Keywords: ABC transporters, placenta, metabolic, hypertensive, pregnancy condition

Introduction: A significant proportion of drugs used in pregnancy for acute, chronic, or preventive purposes are substrates of ABC transporters, which limit the transfer of drugs through the placenta. The integrity of the placental barrier function can often be damaged in complicated pregnancies involving ABC transporter dysregulations and abnormal drug disposition. The most common medical conditions encountered during pregnancy are metabolic and hypertensive disorders, which are characterized by increased drug consumption.

Aims: This study aimed to examine and compare the expression of placental ABCB1 (P-glycoprotein, P-gp) and ABCG2 (breast cancer resistance protein, BCRP) drug transporters in groups with metabolic and hypertensive pregnancy conditions to predict potential changes in drug transport across the placenta.

Methods: Human term placental samples were collected immediately after childbirth from mothers with metabolic or hypertensive pregnancy conditions. Metabolic disorders included pre-gestational obesity (BMI ≥30 kg/m^2, n=12) and gestational diabetes mellitus (n=10) while hypertensive disorders included gestational hypertension (blood pressure ≥140/90 mmHg, n=10) and preeclampsia (n=6) alone. Placental samples from healthy pregnancies (n=12) were used as controls. The mRNA and protein expression of ABCB1 and ABCG2 were determined by RT-PCR and western blot techniques.

Results: The expression changes of each transporter were similar within metabolic or hypertensive groups. The ABCB1 expression decreased by almost half in all conditions and the greatest reduction was observed in preeclampsia compared to controls. ABCG2 expression was doubled in pregnant women with obesity and gestational diabetes mellitus, while remaining unchanged in gestational hypertension and preeclampsia.

Conclusion: Overall, our findings suggest a possible weakening of the protective function of placental ABCB1 in metabolic and hypertensive pregnancy conditions, which may be associated with higher substrate levels in the feto-placental compartment. According to the reduction of ABCB1 in the placenta, the use of drugs associated with ABCB1 transport may warrant careful consideration during pharmacotherapy. At the same time, increased risk may not be assumed for ABCG2 substrate drugs in pregnant women with cardiometabolic disorders.

Source(s) of research support: The study was supported by EKÖP-25-3-SZTE-197 New National Excellence Program of the Ministry for Culture and Innovation from the Source of the National Research, Development and Innovation Fund and „Géza Hetényi" Grant, University of Szeged [No.:5S 724 (A202)].

Ethical Committee Approval: The study protocol was conducted in accordance with the Declaration of Helsinki and approved by the Clinical Research Ethics Committee of the University of Szeged (registration number: 57/2020-SZTE).

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Pregnancy after Breast Cancer Management in a Young Woman: Case Report

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Keywords: breast cancer, pregnancy after cancer, fertility preservation

Introduction: Pregnancy after triple-negative breast cancer (TNBC) has historically been discouraged due to recurrence risk. Recent evidence suggests it may be safe in selected patients´ post-curative treatment.
Aims: To report a successful pregnancy following Stage IIB TNBC treatment and highlight fertility preservation and multidisciplinary care considerations.
Methods: A 30-year-old woman with Stage IIB TNBC underwent neoadjuvant chemotherapy, breast-conserving surgery, radiotherapy, and ovarian suppression for fertility preservation. She conceived spontaneously two years after completing treatment. Pregnancy was monitored by oncology and obstetrics teams.
Results: The pregnancy progressed without complications, resulting in the term delivery of a healthy newborn. Both mother and child remain in good health. Genetic counseling and extended hereditary cancer testing were performed due to the aggressive tumor biology.
Conclusion: Pregnancy after TNBC can be safe when managed within a multidisciplinary framework and after an adequate disease-free interval. Careful oncologic follow-up and genetic assessment are recommended.

Source(s) of Research Support: No financial support or grants were received.
Ethical Committee Approval: Written informed consent for publication was obtained; institutional ethical standards were followed.
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The Benefits and Outcome of Prenatal Whole Exome Testing (WES) in a Romanian Cohort

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Keywords: prenatal diagnosis, genetic testing, genetic counseling, whole exome sequencing
Introduction: Prenatal genetic testing has become a very important tool for pregnant families, that helps the multidisciplinary medical team and the family prepare accordingly for the specific genetic disorder. The recommendations for prenatal genetic diagnostic testing are known family history of genetic disease, ultrasound structural fetal abnormality, high-risk screening test.
Aims: The scope of the present study is to assess the clinical utility of adding prenatal whole exome sequencing (WES) to a Romanian cohort of pregnant patients. When other classical genetic studies have been conducted, such as conventional chromosomal analysis and array CGH and have not yielded a genetic explanation for the specific recommendations, such as high screening results, multiple malformations in the ultrasound examination, or known family history of genetic variation, we evaluate the benefits WES brings to such patients.
Methods: Patients consents were obtained after explaining limitations and benefits of the test. Using Massive Parallel Sequencing of the coding region of the human genome on Next Generation Sequencing (NGS) Platform, NovaSeq 6000 (average coverage > 100×, read length: 2 × 100 bp). The Twist Human Core Exome kit RefSeq & Mitochondrial panel (Twist Bioscience, San Francisco, CA, USA) was used for the library preparation. Bioinformatic analysis was performed by direct comparison of the genome of the test sample with the human reference sequence (hg38).
Results: We report 10 cases of prenatal WES testing in pregnant mothers from the Romanian population between 2022 and 2023 from a private laboratory in Bucharest, Romania. 50% of the selected cohort was evaluated through prenatal WES testing and were positive for genetic variation. All the medical data was interpreted and transmitted to the families and to the multidisciplinary medical team in order to better manage the cases, through personalised care. The selected cohort contains patients with clinical diagnosed disorders that can have a genetic component, pregnancies at risk – after the ultrasound evaluation, presenting with different malformations such as central nervous system malformations, arrhythmias, cardiac malformation, intrauterine growth restriction, renal malformations.
Conclusion: The high yield of 50% of genetic diagnosis in our selected cases - a similar percentage to other published international studies, should highlight the importance of this state-of-the-art test. Based on the particular findings of each case the medical team could personalize and target the management of the pregnancies and better understand the overall outcome. The families can make informed decisions based on the genetic results and also can get genetic counseling for the recurrence risk for next pregnancies. Prenatal WES is a comprehensive genetic tool that helps the family. It provides a wider picture of the respective fetus and should be considered in selected cases.
Source of research support: none
Ethical Committee Approval: Ethical Committee Personal Genetics, 01/01.02.2022.
Acknowledgements: We thank Cedars-Sinai Medical Center's International Research and Innovation in Medicine Program, and the Regional Cooperation for Health, Science and Technology Association (RECOOP HST Association) for their support.

Impact of Prenatal Stress and Cigarette Smoke Exposure on Early Neurobehavioral Development in Rats: A Combined DOHAD Perspective

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Keywords: prenatal, stress, smoking, neurobehavioral development

Introduction: Maternal stress and cigarette smoke exposure are critical environmental factors influencing early neurodevelopment.
Aims: To determine how prenatal stress at different gestational periods and exposure to tobacco smoke during pregnancy influence early neurobehavioral development in rats.
Methods: Pregnant rats were exposed to whole-body smoke exposure twice daily from mating until delivery, or to restraint stress twice daily in different periods of pregnancy. After delivery offspring were tested daily for somatic growth, maturation of facial characteristics and neurobehavioral development until 3 weeks of age.
Results: Critical physical reflexes indicative of neurobehavioral development (eyelid reflex, ear unfolding, $p<0,01$) appeared significantly later in pups exposed to cigarette smoke prenatally, this also resulted in delayed appearance of reflexes indicating neural maturity (hind limb grasping, fore limb placing reflexes, $p<0,05$). Mid-pregnancy stress resulted in a subtle faster development in the appearance of eyelid and auditory startle reflexes and in the disappearance of crossed extensor reflexes. Pups exposed to stress in the last week of intrauterine life showed a delay in air righting and a slight enhancement in the appearance of auditory startle.
Conclusion: Although the effects of cigarette smoke were modest, both exposures support the concept that early-life adversities shape developmental trajectories in accordance with the Developmental Origins of Health and Disease (DoHAD) perspective. Together, the findings highlight that even mild prenatal insults can program neurobehavioral vulnerabilities, emphasizing the importance of minimizing maternal stress and smoking during pregnancy to support optimal brain development.

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Placental Alpha Microglobulin-1 (PartoSure, AmniSure) Tests for the Prediction of Preterm Birth and Premature Rupture of Membranes

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Keywords: PAMG-1; placental alpha microglobulin-1; cervical length, preterm birth

Introduction: Threatening preterm birth (TPB) is a major cause of hospitalisation in the third trimester of pregnancy. An early diagnosis is crucial for reducing perinatal mortality and morbidity. Current diagnostic criteria are based on symptoms and short cervical length (CL). However, there is a high false-positive rate when using these criteria, which implies overtreatment. PartoSure is an immunoassay that measures the presence of placental alpha macroglobulin-1 (PAMG-1), a protein present in the amniotic fluid throughout pregnancy. Cervicovaginal detection of PAMG-1 in a patient with unruptured membranes is associated with an increased likelihood of delivery in the next 14 days. In cases of preterm premature rupture of membranes (PPROM) AmniSure is available to detect PAMG-1 vaginally.
Aims: To evaluate the combined identification of PAMG-1 in cervicovaginal secretions and ultrasound cervical length measurement in pregnant women with symptoms of TPB and intact or ruptured amniotic membranes to predict the time of labor onset.
Methods: A prospective study including 55 pregnant women at gestational age of 24–37 weeks with symptoms of PB. Presence of PAMG-1 was determined using the commercially available PartoSure test kit (Parsagen Diagnostics Inc, Boston, USA) and the cervical length was measured using transvaginal ultrasound and considered shortened under 30mm.
Results: Among the 55 subjects included, 31 complained of PPROM, AmniSure was negative in 18 cases of which 15 patients delivered after 2 weeks or later. All the 13 patients with positive AmniSure results delivered within 2 weeks (Sensitivity: 100%, Specificity: 87%). Among the 24 patients with TPB but without PPROM Partosure was negative in 21 cases and 20 of them delivered after 2 weeks or later, All the 3 patients with positive Partosure tests delivered within 2 weeks (Sensitivity: 96%, Specificity: 96,6%).
Conclusion: Using the combination of ultrasound cervicometry and PAMG-1 testing in women with symptoms of PB and the cervical length under 30 mm may allow to identify those with a high risk of PB within 7 and 14 days more accurately, may lead to more targeted treatment, thus reducing unnecessary hospitalization and avoiding unnecessary treatment.

Ethical committee Approval: Regional Ethical Committee Approval (reference number: 32/2014-SZTE).
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Infections and Immunology (IFI)

Micellium

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Pediatric COVID-19 and MIS-C: Unseen Endocrine Shifts

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Keywords: endocrine response, COVID-19, MIS-C, children

Introduction: Endocrinopathies in the context of COVID-19 and MIS-C should remain a focus for clinicians, given both the direct impact of SARS-CoV-2 on endocrine structures, mediated by ACE2 receptors, and the effects of systemic inflammation on hormone production.
The study aims to evaluate alterations in the hormonal profile of patients with COVID-19 and MIS-C, and to determine their association with disease severity and genetic markers.
Methods: We examined 123 children with COVID-19, 32 patients with MIS-C, and 25 SARS-CoV-2-negative controls. Thyroid-stimulating hormone (TSH), free T3 (FT3), free T4 (FT4), luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol (E2), and total testosterone (T) were measured in all participants. Growth hormone (GH) and insulin-like growth factor-1 (IGF-1) were assessed in 90 cases. In 75 patients, the *ACE2 rs2074192* gene polymorphism was analyzed. Statistical analysis was performed using IBM SPSS Statistics 21.0. Statistical significance was set at $p<0.05$.
Results: The prevalence of euthyroid sick syndrome (ESS) increased with disease severity: 9.09% in mild COVID-19, 25.00% in severe cases, and 42.86% in MIS-C ($\chi^2=19.82$, $p<0.001$). Low FSH levels were identified in 13.43% of children with COVID-19 and in 25.00% of MIS-C cases, while elevated FSH concentrations were observed in 18.75% of prepubertal MIS-C patients ($p<0.05$). Among adolescents with MIS-C, reduced FSH levels were observed in 31.25%, although no such findings were observed in the control group ($p<0.05$). Testosterone levels were significantly lower in children with COVID-19 and MIS-C than in controls ($p<0.05$), whereas E2 levels did not differ between groups ($p>0.05$). GH levels remained within reference ranges in all participants, whereas IGF-1 was reduced in 30% of children. The frequency of low IGF-1 increased with disease severity: 12.5% in mild, 25% in moderate, 53.3% in severe COVID-19, and 66.7% in MIS-C ($\chi^2=22.42$, $p<0.001$). Carriers of the *ACE2 rs2074192* T allele showed significantly lower GH, IGF-1, TSH, FT3, and FT4 levels compared with C-allele carriers.
Conclusion: Hormonal assessment is not routinely included in the primary diagnostic examination for pediatric COVID-19 and MIS-C, leaving many endocrine disturbances unrecognized. Endocrine dysfunction should be considered by pediatricians, infectious disease specialists, and endocrinologists. Timely evaluation and monitoring of hormonal status may help prevent long-term complications.
Source(s) of research support: This research was partially funded by RECOOP Fusion Grant # 030 “COVID-19 Severity and Gene Polymorphism in Children and Adults”.
Ethical Committee Approval: The Bioethics Committee of I. Horbachevsky Ternopil National Medical University approved this study (Protocol No. 71, dated 25 October 2022).
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Investigation of EV-DNA in Juvenile Idiopathic Arthritis

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Keywords: extracellular vesicles, cell-free DNA, autoimmune diseases

Introduction: Small extracellular vesicles (sEVs) are small, membrane-bound particles released by cells into all body fluids and play an essential role in intercellular communication or biomarker discovery. They can be one of the forms of extracellular DNA (EV-DNA) protecting it from degradation in circulation by nucleases.
Aims: Our hypothesis is that EV-DNA and sEVs can act as damage associated molecular patterns (DAMPs) in various diseases including autoimmune diseases such as juvenile idiopathic arthritis (JIA).
Methods: sEVs were isolated from plasma and synovial fluid (SF) obtained from healthy (CTRL) and JIA patients by size exclusion chromatography. sEVs were characterized by transmission electron microscopy, nanoparticle tracking analysis and western blot. DNA was extracted using a commercial kit and further analysed.
Results: No significant differences were observed between DNA concentration of CTRL and JIA plasma- or SF-derived sEVs. JIA SF-derived sEVs exhibited a higher proportion of DNA associated with the vesicles surface, which may contribute to increased vesicle size and a propensity for aggregate formation. These samples have only 10% of DNA protected within the vesicle lumen. In contrast, CTRL SF-derived sEVs contain about 55% of surface-associated DNA and 45% intravesicular DNA. Plasma-derived sEVs from both CTRL and JIA patients contain approximately 30 – 45 % of DNA located on the surface and 55 – 60% protected inside vesicles. Nuclear and mitochondrial DNA was detected in both CTRL and JIA sEVs, with a higher copy number of mitochondrial DNA protected against DNase I.
Conclusion: This study demonstrates a protected presence of DNA including mtDNA in plasma-derived or SF-derived sEVs from both CTRL and JIA patients. Depending on its localisation, when EV-mtDNA is exposed, it could act as DAMP and potentiate inflammatory responses in JIA, whereas intravesicular mtDNA can be protected from pattern-recognition receptors, thereby preventing immune activation.

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Ethical Committee Approval: Ethics Committee of the National Institute of Children's Diseases at the Faculty of Medicine, Comenius University, Bratislava, Slovakia, under number EK 218/2020.
Acknowledgements: We thank Cedars-Sinai Medical Center's International Research and Innovation in Medicine Program, and the Regional Cooperation for Health, Science and Technology Association (RECOOP HST Association) for their support.

Seasonal Dynamics of Childhood Community-Acquired Pneumonia Admitted at Lviv Infection Diseases Clinic: Marked Surge and Shifting Patterns in 2024–2025

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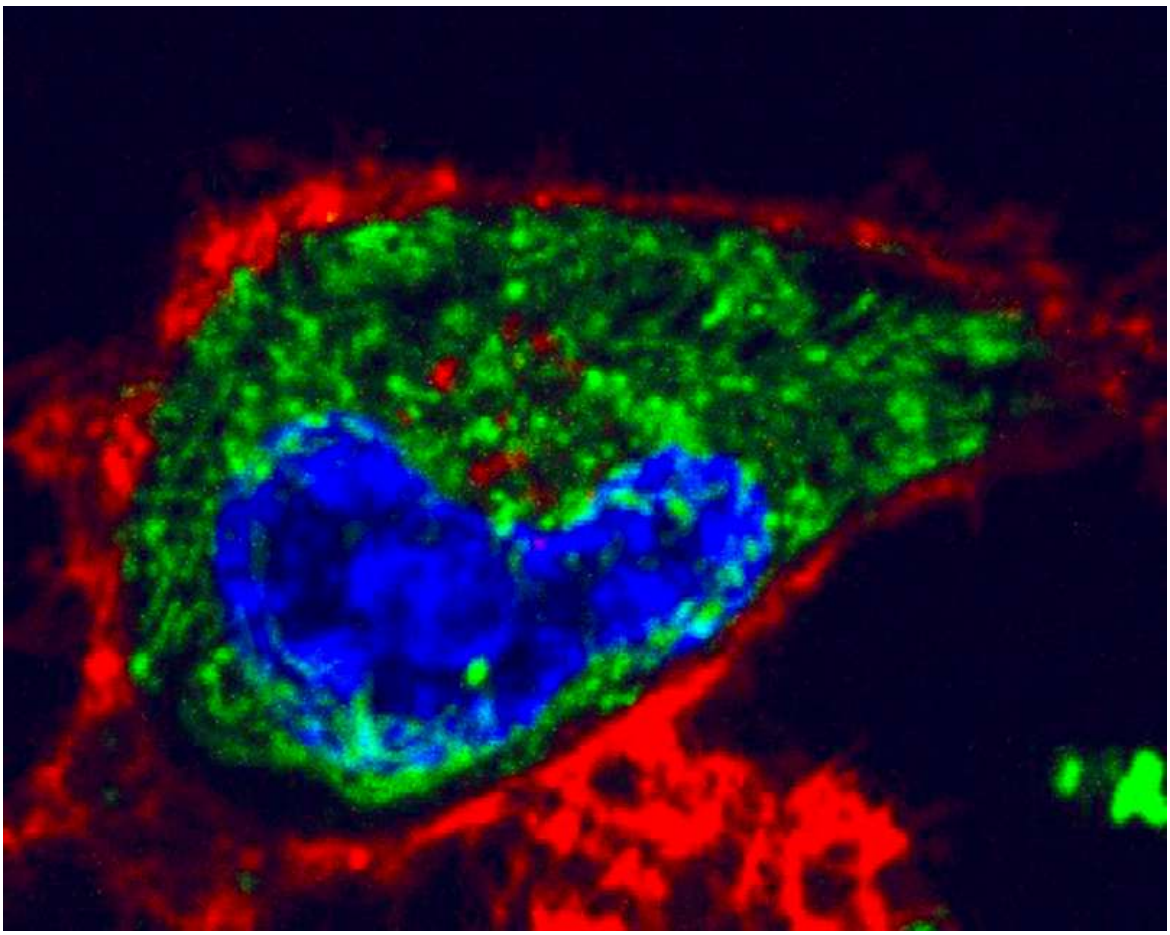
Keywords: community-acquired pneumonia, children, post-COVID, immunity debt

Introduction: The COVID-19 pandemic and associated non-pharmaceutical interventions dramatically suppressed circulation of common respiratory viruses, creating an "immunity debt" in children. The post-COVID era has been marked globally by intense rebound epidemics of RSV, influenza, metapneumovirus, and parainfluenza.
Aims: This study investigates whether the unprecedented Community-acquired pneumonia (CAP) surge and striking clinical shifts observed in 2024–2025 represent delayed consequences of this disrupted viral ecology.
Methods: Retrospective cohort study of all pediatric CAP admissions in a single clinic - Lviv Infection Diseases Hospital (2023–Q3 2025). Total quarterly admissions were recorded throughout; clinical data (age, etiology, lab, X-ray & ultrasound results) were available for Q4 2024 (n=50/78) and Q1 2025 (n=122/128) patients. Statistical comparisons used χ^2 or Fisher's exact test and Mann–Whitney U test; odds ratios with 95% CI were calculated.
Results: In the post-COVID years, CAP admissions increased explosively: 58 cases in the whole of 2023 → 155 in 2024 → record 128 cases in Q1 2025 alone (>7-fold increase vs 2023 baseline; 64% higher than the previous peak quarter Q4 2024 with 78 cases). Age distribution remained stable (median 6 years Q4 2024 vs 5 years Q1 2025, p=0.42; infants <1 year: 10% vs 11.3%, p=0.74). A highly significant and near-complete reversal of laterality occurred within one quarter: - Pure right-sided segmental pneumonia rose from 8% (4/50) in Q4 2024 to 50% (31/62) in Q1 2025 (p<0.0001; OR 11.8, 95% CI 4.3–32.4); - Left-sided segmental pneumonia fell from 74% (37/50) to 26% (16/62) (p<0.0001); - Bilateral involvement remained similar (18% → 24%, p=0.49). Upper-zone involvement increased from 2% (1/50) to 18% (11/62) (p=0.004) and was observed almost exclusively in right-sided viral cases. Lower-zone predominance persisted (>80%) in both periods. Documented viral etiology doubled from 26% (13/50) to 48% (30/62) (p=0.019); bacterial etiology declined from 56% to 37% (p=0.046). Among right-sided cases in Q1 2025, 61% were viral compared with only 25% in Q4 2024.
Conclusions: In the post-COVID period, this clinic experienced the largest CAP epidemic on record, accompanied by a statistically dramatic inversion of laterality from left/bilateral to right-sided disease and increased upper-zone involvement – changes tightly correlated with viral predominance. These findings strongly support the "immunity debt" hypothesis: reduced viral exposure during the pandemic created a large susceptible pediatric cohort, leading to intense rebound epidemics with classic viral clinical signatures. Right-sided CAP (OR >11) and upper-zone localization emerged as powerful, rapid bedside markers of viral rather than bacterial etiology.
Source(s) of research support: No research support was received for this work
Ethical Committee Approval: The study was conducted as part of the scientific work of the department (state registration number 0124U000805). DHLNMU Ethical Committee, protocol No. 1, January 15, 2025.
Acknowledgements: The authors express gratitude to the Administration of the Lviv Infectious Disease Clinical Hospital for providing access to archived medical records. We thank Cedars-Sinai Medical Center's International Research and Innovation in Medicine Program, and the Regional Cooperation for Health, Science and Technology Association (RECOOP HST Association) for their support.

Nanomedicine and Safety (NMS)



Cellural Valentine

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Sub-chronic Copper Oxide Nanoparticle Inhalation Alters Adaptive and Innate Immunity in Mice

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Key words: zinc oxide nanoparticles, immunotoxicity

Introduction: Copper oxide nanoparticles (CuO NPs) are increasingly employed across industrial applications, and their use as antimicrobial agents in medical settings further contributes to human exposure. Concerns have been raised about their potential toxicity, including effects on the immune system and blood, yet available data on their immunotoxicity remain limited.
Aims: This study aimed to assess immune and inflammatory responses in mice following continuous 6-week inhalation exposure to CuO NPs (number concentration 1.40×10^6 particles/cm³; geometric mean diameter 20.4 nm; mass concentration 32.5 μg CuO/m³).
Methods: Immune function was evaluated using assays examining human lymphocyte proliferation, leukocyte phagocytic activity, respiratory burst, and in vitro cytokine production.
Results: CuO NP inhalation significantly enhanced T-lymphocyte proliferative responses to mitogenic stimulation, as well as basal splenocyte proliferation. Exposure markedly increased production of IL-12p70, the Th1 cytokine IFN-γ, and the Th2 cytokines IL-4 and IL-5, while TNF-α and IL-6 levels remained unchanged. Granulocyte phagocytic activity was significantly reduced, accompanied by a slight decrease in respiratory burst, whereas monocyte phagocytosis was unaffected. The proportions of CD3+, CD3+CD4+, CD3+CD8+, and CD3−CD19+ cell subsets in the spleen, thymus, and lymph nodes showed no differences between exposed and control groups. Hematological parameters also remained unchanged.
Conclusion: These findings suggest that sub-chronic inhalation of CuO NPs leads to undesirable modulation of immune function. Activation of adaptive immunity was reflected in enhanced lymphocyte proliferation and cytokine secretion, with evidence of both Th1 and Th2 pathway activation. Innate immunity was negatively impacted, as indicated by impaired granulocyte phagocytic function.
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Ethical Committee: Animal Ethics Committee of the Institute of Analytical Chemistry of the Czech Academy of Sciences (55/2015).
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Theranostic Upconversion Nanoparticles for Cancer Treatment

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Keywords: upconversion, nanoparticles, photodynamic therapy, theranostic

Introduction: Cancer remains a major threat to human health, causing millions of deaths annually. Photodynamic therapy (PDT) is a clinically applied modality and a promising alternative for treating unresectable or therapy-resistant tumors. To enhance PDT efficacy and selectivity, upconversion nanoparticles (UCNPs) with the required functional properties allow the development of a highly selective theranostic PDT platform that integrates therapy and diagnostics.
Aims: The investigation of new core-shell UCNPs (CS-UCNPs) with covalently conjugated photosensitizers (PSs) for bimodal imaging and PDT of aggressive cancers.
Methods: Core-shell UCNPs codoped with various lanthanide and transition metal ions were prepared by high-temperature coprecipitation method and modified by biocompatible poly(*N,N*-dimethylacrylamide)-based (PDMA) polymers. The clinically used verteporfin (VP) and temoporfin (mTHPC) were covalently conjugated to the surface of polymer-coated CS-UCNPs. The particle morphology, colloidal stability, and luminescent and magnetic properties were thoroughly characterized. Biocompatibility, contrast properties and photodynamic activity of the CS-UCNPs conjugated with VP/mTHPC to PaTu-8902 pancreatic adenocarcinoma and U-251 MG glioblastoma cell lines were studied *in vitro* and *in vivo*.
Results: First, Fe^{2+}-doped UCNPs enhanced upconversion luminescence and efficiently transferred energy to PSs, resulting in highly efficient PDT against human Capan-2 pancreatic adenocarcinoma. Here, UCNPs were further modified by introducing $NaHoF_4$ or $NaYF_4$ shell to enable bimodal optical and MR imaging. Covalent VP and mTHPC binding to the polymer-coated CS-UCNPs provided colloidally stable conjugates with high ROS generation at 808 and 980 nm excitation. *In vitro*, these conjugates effectively eliminated human U-251MG glioblastoma and PaTu-8902 pancreatic adenocarcinoma cells under NIR irradiation. *In vivo*, NIR-induced PDT with VP-conjugated CS-UCNP@PDMA particles produced local tumor damage and a clear antitumor effect in pancreatic adenocarcinoma.
Conclusion: Integration of luminescence with magnetic properties in CS-UCNPs demonstrated their applicability for multimodal bioimaging and theranostic treatment. VP- and mTHPC-conjugated CS-UCNP@PDMA particles constitute a versatile platform for NIR-induced PDT, paving the way for future research and clinical applications.

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Ethical Committee Approval: The biological experiments were ethically reviewed and performed under the European directive 86/609/EEC and approved by the Ministry of Education, Youth, and Sports of the Czech Republic (MSMT-2309/2018-4).
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Polymer-Modified Upconverting Nanoparticles for Multimodal Imaging and Hypericin Delivery

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Keywords: nanoparticles, upconversion, multimodal imaging, drug delivery system

Introduction: Nanomaterials offer significant potential for biomedical technologies, including bioimaging, drug delivery, and photodynamic therapy (PDT), due to their ability to provide minimally invasive, localized, and patient-friendly treatments. The combination of nanocarrier-based delivery systems with optical imaging significantly increases their effectiveness, enabling real-time monitoring of drug accumulation and treatment progression. Upconversion nanoparticles (UCNPs), which convert near-infrared (NIR) excitation into visible emission, are especially appealing for this purpose due to their high photostability, minimal autofluorescence background, and deep NIR light penetration into tissue.
Aims: The development of polymer-coated core-shell UCNPs (CS-UCNPs) for multimodal imaging and delivery of hypericin photosensitizer for cancer treatment.
Methods: $NaYF_4$:Yb,Er@$NaYF_4$:Nd CS-UCNPs were prepared by the high-temperature coprecipitation method and modified with biocompatible poly(*N*,*N*-dimethylacrylamide-*co*-2-aminoethyl acrylate)-alendronate (P(DMA-AEA)-Ale). Hypericin (Hyp), a natural carbopolycyclic compound used as a photosensitizer, was then conjugated to the surface of polymer-coated particles. The morphology, colloidal stability, and luminescent properties of CS-UCNP@P(DMA-AEA)-Ale-Hyp nanoparticles were thoroughly characterized. Hyp delivery was investigated, and multimodal intracellular visualization was performed using fluorescence imaging, upconversion luminescence, and Raman microspectroscopy.
Results: Introduction of a $NaYF_4$:Nd shell on $NaYF_4$:Yb,Er particles enhanced upconversion luminescence and prevented surface quenching. P(DMA-AEA)-Ale coating ensured colloidal stability of nanoparticles and their biocompatibility. Hyp release was much more pronounced in the acidic environment than in the neutral one, which is beneficial for PDT. In Jurkat cells, a tumor tissue model, CS-UCNP@P(DMA-AEA)-Ale-Hyp particles were much more effective than pure Hyp for PDT and enabled multimodal cell tracking.
Conclusion: A combination of luminescence with drug delivery abilities of polymer-coated CS-UCNPs demonstrated their applicability for multimodal cell imaging and cancer treatment, and great potential for *in vivo* applications. Hypericin-conjugated CS-UCNP@PDMA particles represent a versatile platform for effective photosensitizer delivery and enhanced photodynamic therapy.
Research was supported by the Czech Science Foundation (No. 24-10125S).
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Multicore Nanoassemblies for Enhanced Magnetic Hyperthermia

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Keywords: nanoparticles, drug delivery systems, cancer therapy, magnetic hyperthermia

Introduction: Magnetic hyperthermia is an alternative cancer therapy that requires nanoparticles (NPs) with high heating efficiency, whereas single-core iron oxide NPs often produce insufficient energy. Multicore nanoassemblies (NAs) offer a new platform for enhanced heat generation. Flash nanoprecipitation allows to encapsulate NPs and hydrophobic anticancer gossypol (GS) withing chitosan (CS) matrix to create NAs capable of targeted drug delivery and magnetic hyperthermia.

Aims: To develop NAs with enhanced heat generation compared to single-core NPs, providing a versatile platform for combined cancer therapy.

Methods: Monodisperse spherical and cubic NPs of different diameters were synthesized by thermal decomposition. NAs were produced by rapid mixing an aqueous CS phase with an organic GS/NPs phase using confined impingement jet mixer. Characterization included transmission electron microscopy (TEM), dynamic light scattering (DLS), and magnetic hyperthermia (29.5 kA/m; 486 kHz).

Results: TEM confirmed the formation of uniform spherical (5, 8, 10 nm) and cubic NPs (edge = 19 nm). DLS revealed corresponding hydrodynamic diameters D_h = 69, 68, 50 for spherical, and 97 nm for cubic NPs-based NAs. All NAs exhibited substantially higher specific absorption rates (SAR) compared to their corresponding single NPs. For spherical systems, SAR increased from 7.1±1.5 to 46.5±7.2 W/g for 5 nm NPs, from 15.7±6.7 to 88.6±14 W/g for 8 nm NPs, from 3.6±0.3 to 36±10 W/g for 10 nm NPs. Likewise, cube-based NAs showed an increase from 400±22 to 504±95 W/g. NAs composed of spherical NPs demonstrated the strongest enhancement in heating performance (5-10x) which we attribute to their tighter packing and more pronounced collective magnetic interactions. In contrast cube-based NAs exhibited only modest increase (≈1.3x), likely due to reduced coupling and the presence of fewer cubes per as assembly as observed by TEM, although they still displayed the highest SAR values overall.

Conclusion: The multicore NAs significantly outperform individual NPs in heat generation and offer a promising platform for integrating magnetic hyperthermia with targeted drug delivery. This comparison with single NPs has not been presented before, highlighting the novelty of our system, which uniquely combines enhanced hyperthermia and potential targeted drug delivery. Further optimization will facilitate the development of effective nanoplatform for cancer therapy.

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Neuro-Safe Biochar from Agricultural Waste

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Keywords: agricultural waste, biochar from sunflower seed husk, neurotoxicity risk, brain nerve terminals

Introduction: Usage of agricultural waste for synthesis of carbon materials based on "green" principles is a very promising stream in modern biotechnology as well as environmental sciences and management. Search of efficient biocompatible sorbents, which possess zero neurotoxicity is a relevant task in current research. Biochars are very promising carbon materials for adsorption of heavy metals in the environment, waste water and also in human organisms.

Aims: The aim of this study was to assess neurotoxicity risk of biochar synthesized from sunflower seed husk (SFB).

Methods: SFB was synthesized by pyrolysis at 800°C without special functionalization. Neurotoxicity risk of SFB was assessed in animal model using presynaptic nerve terminals (synaptosomes) isolated from cortex of Wistar rats according to the method of Cotman (1974). [^{3}H]GABA and [^{3}H]glutamate transportation in nerve terminals was monitored using a radiolabelled assay. The fluorimetric experiments were carried out using a dye JC-1.

Results: The changes in the extrasynaptosomal level of the neurotransmitters reflect the plasma membrane disintegration and their impaired transportation. It was shown in radiolabelled experiments that SFB did not change the synaptosomal ambient levels of the excitatory neurotransmitter L-[^{3}H] glutamate and inhibitory one [^{3}H] GABA within the concentration range 0.25-1 mg/mL. In the fluorimetric experiments using the dye JC-1, SFB (1 mg/mL) did not change the mitochondrial membrane potential of the nerve terminals, and so SFB did not depolarize the mitochondria membrane. These fluorimetric data completely corresponded to the above results obtained using L-[^{3}H] glutamate and [^{3}H]GABA.

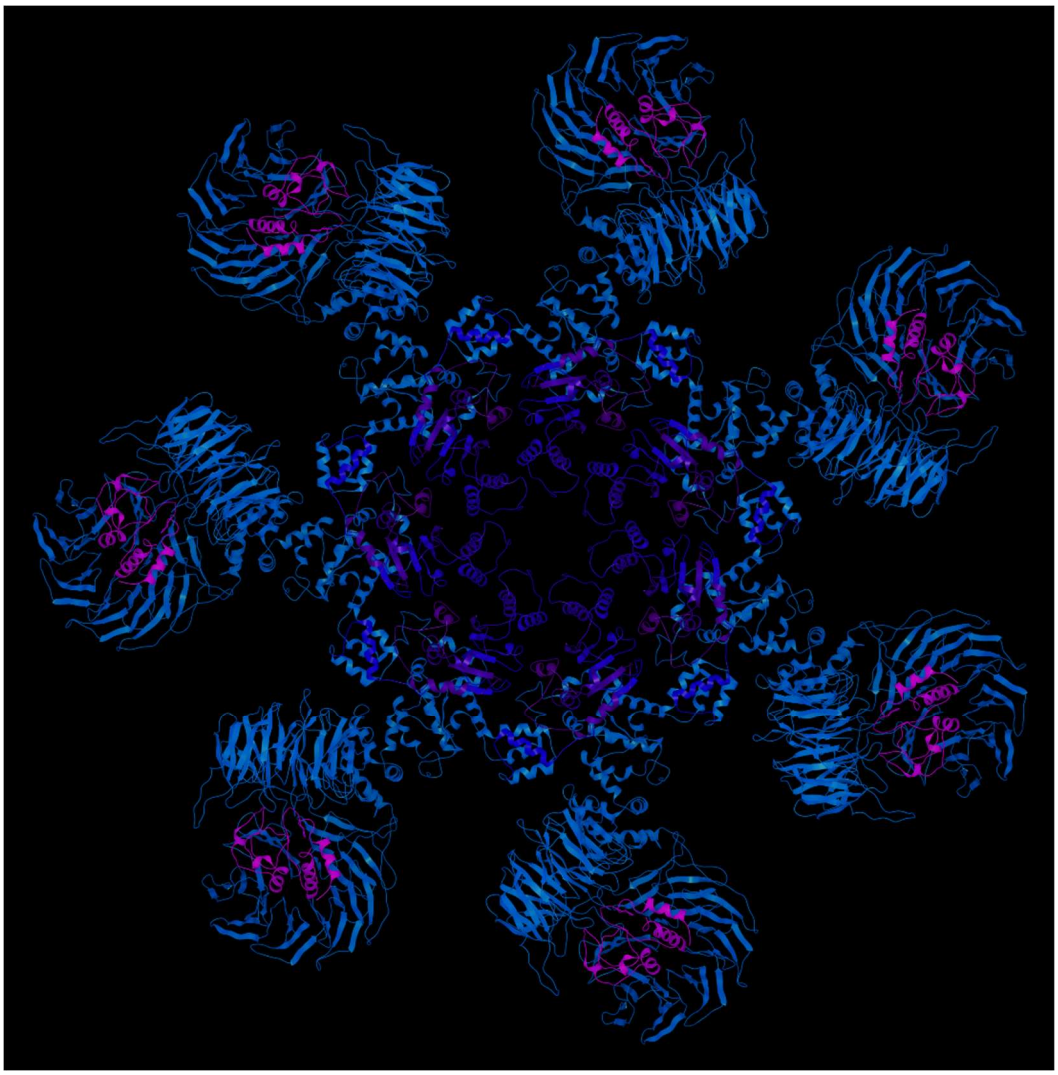
Discussion and Conclusion: SFB demonstrated absence of neurotoxicity signs, and so high biocompatibility, and therefore SFB has perspectives to be used as adsorbent in biotechnology and medicine.

Ethical approval: The experimental procedures were conducted according to "Scientific Requirements and Research Protocols" and "Research Ethics Committees" from the Declaration of Helsinki. Experimental protocols were approved by the Animal Care and Use Committee of the Palladin Institute of Biochemistry (Protocol # 1 from 10/01/2024).

Funding: This work was supported by the National Research Foundation of Ukraine: NRFU grant # 2023.03/0036 «Functionalized carbon-based nanomaterials obtained by "green methods" from Ukrainian agricultural waste to combat multilevel neurotoxicity induced by xenobiotic/essential transition metals and arsenic». PI- N. Krisanova.

Acknowledgements: The study was supported by Cedars-Sinai Medical Center's International Research and Innovation in Medicine Program and the Regional Cooperation for Health, Science and Technology Association (RECOOP HST Association) and the participating Cedars-Sinai Medical Center - RECOOP Research Centers (CRRCs).

Clinical and Epidemiological Research (CER)



"The Final Flower" – model of the human apoptosome

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Technology Acceptance as a Psychosocial Determinant in the Telerehabilitation of Patients with Metabolic Syndrome

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Keywords: metabolic syndrome, modified UTAUT2, patient behavior, technology acceptance, telerehabilitation

Introduction: The advent of telerehabilitation has created new opportunities for the care of patients with metabolic syndrome. In distant rehabilitation, technology acceptance is particularly important because home-based project is based on digital devices and many patients are less familiar with the use of them.
Aims: Our aim was to explore technology acceptance among patients undergoing a three-month complex, telemedicine-supported metabolic rehabilitation. We were curious to see how different factors influence the intention to use rehabilitation technologies and how this changes through the rehabilitation process.
Methods: A paper-pencil questionnaire survey was administered on the last day of the preparatory week of the rehabilitation programme (T1) and at the follow-up visit after the closing (T2). 145 patients participated in the survey, which measured the impact of several latent variables on the intention to use rehabilitation devices. Structural equation modelling with the least squares method (SEM-PLS) was used to explore the relationships among variables. Respondent segments also were identified by a hierarchical cluster analysis.
Results: Facilitating conditions have the greatest impact on the behavioral intention (BI) to use technology. Effort expectancy has no direct effect on BI, only through performance expectancy which may be due to the fact that in telerehabilitation settings patients are rather goal-driven than experience-driven. By comparing the T1 and T2 datasets, we have provided empirical support for an old hypothesis: the experience of using the tool for a time has led to a significant reduction in the impact of facilitating conditions and a corresponding increase in the dominance of performance expectancy. Based on the attitudes of the respondents we found three clusters: "enthusiastic users" (50.3%), "liberated adopters" (32.4%) and "distrustful reluctants" (17.3%).
Conclusion: This behavior-based functional approach enables treatments to be tailored to actual technology usage demands rather than presumptive societal features. So, before beginning rehabilitation, attempts should be undertaken to identify patients' clusters in clinical practice and rehabilitation should be planned according to individual's attitude towards technology.

Ethical Committee: Hungarian Sci. and Research Ethics Committee, BM/25016-1/2024.
Acknowledgments: We thank Cedars-Sinai Medical Center's International Research and Innovation in Medicine Program and the Regional Cooperation for Health, Science and Technology Association (RECOOP HST Association) for their support.

Preferred Features of Continuous Glucose Monitoring Sensors: A Conjoint Analysis-Based User Preference Study

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Keywords: Continuous glucose monitoring, conjoint analysis, patient preferences, diabetes mellitus

Introduction: Continuous glucose monitoring (CGM) systems improve diabetes management by providing near real-time glucose data and supporting therapeutic decisions. Device adoption, however, depends strongly on user-valued features. This study examines which technological attributes patients and caregivers prioritise when selecting a CGM system.
Aims: To quantify user preferences for CGM devices and identify the technical and usability-related features that drive decision-making. We also compared subgroups, including parents of minors, experienced versus prospective CGM users, and insulin pump users, to reveal preference heterogeneity relevant for clinical guidance and device development.
Methods: An adaptive choice-based conjoint design was used with 824 respondents (79% T1DM). Six attributes were evaluated: data display, accuracy, device language, technical support, sensor lifespan, and calibration frequency. Relative attribute importance and part-worth values were calculated to model subgroup differences.
Results: Data display format and accuracy were the most influential features. Parents of children with diabetes showed lower tolerance for reduced accuracy or limited support. Prospective users were more open to trade-offs than experienced users. Higher-educated respondents placed less emphasis on native-language devices, while insulin-pump users strongly preferred seamless integration.
Conclusion: Sensor lifespan and calibration frequency were secondary factors, whereas accuracy and real-time data access primarily shaped preferences. These findings indicate that future CGM development should prioritise precision, data visualisation, and integration.

Financial Support: This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.
Ethical Committee Approval: The study was approved by the Medical Research Council – Scientific and Research Ethics Committee (BM/28185-3/2024).
Acknowledgments: We thank Cedars-Sinai Medical Center's International Research and Innovation in Medicine Program, and the Regional Cooperation for Health, Science and Technology Association (RECOOP HST Association) for their support.

Relationship Between Nutritional Status and Psychological Distress in Medical Students in Slovakia

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Keywords: body mass index, body composition, neuroticism, anxiety, psychological stress

Introduction: In response to academic demands and overwhelming workload, medical students, despite their advanced knowledge of health and prevention, often adopt unhealthy lifestyle habits. This might negatively affect their physical health and further challenge their mental well-being. The relationship between psychological distress and weight status appears complex and bidirectional, involving biological, psychological, and social pathways.
Aims: The aim of this study was to examine the relationship between nutritional status and indicators of negative affect among medical students in Slovakia.
Methods: Data from 637 (68.8% female) students in their 2nd year of study were collected. Body mass index was calculated from body height and body mass; percent of body fat and visceral fat was assessed by bioimpedance method using InBody 370S device. Levels of neuroticism, anxiety, perceived long-term stress and current stress were assessed using questionnaires.
Results: Based on body mass index, 4.2% of students were classified as obese, 19.5% as overweight, and 6.9% as underweight. Notable inconsistencies emerged between BMI-based categorizations and classifications derived from body composition indicators, with clear gender-specific differences. Overall, higher psychological scale scores tended to align with increasing body mass, body fat percentage, and visceral fat level, although the strength of statistical significance varied. Among females, these trends were more pronounced across BMI categories, whereas in males, body fat percentage and visceral fat level provided more meaningful differentiation. Students in the underweight category also demonstrated slightly elevated psychological scores, though these differences were not statistically significant.
Conclusion: Our findings highlight the importance of gender-specific approaches and the inclusion of body composition analysis in evaluating nutritional status. Both conditions diverging from standard nutritional status - underweight and overweight/obesity - may be linked to psychological distress, highlighting the complex interplay between physical and mental well-being.

Source(s) of research support: -
Ethical Committee Approval: Ethical committee of the Comenius University Faculty of Medicine, and the University hospital in Bratislava, Slovakia, 82/2023, 07.08.2023
Acknowledgements: We thank Cedars-Sinai Medical Center's International Research and Innovation in Medicine Program, and the Regional Cooperation for Health, Science and Technology Association (RECOOP HST Association) for their support.

Spectrum and Frequency of Wheezing Causes in Toddlers

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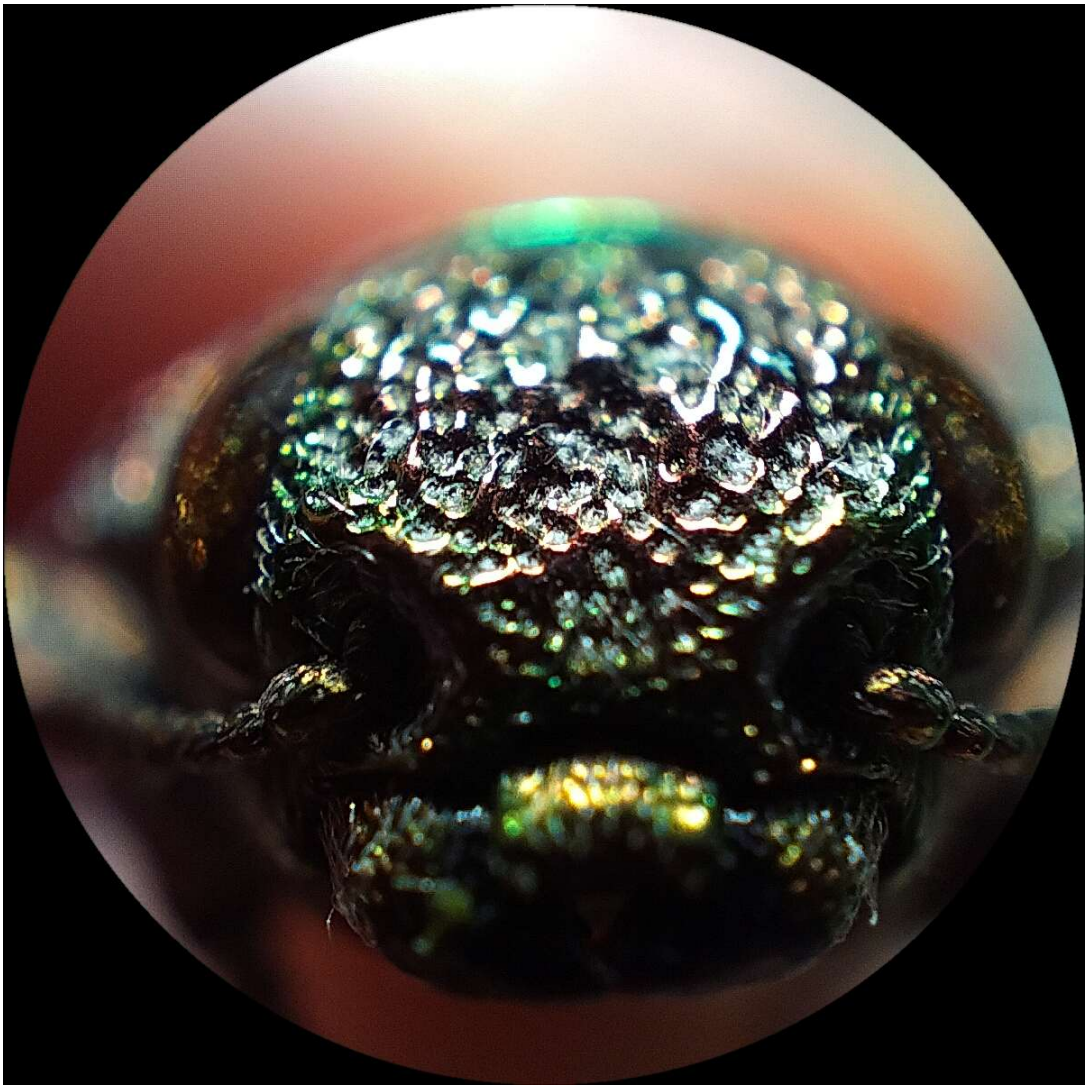
Keywords: wheezing, toddlers, preschool children, etiology, differential diagnosis

Introduction: Wheezing is a common heterogeneous clinical symptom and is more prevalent in early childhood. Its etiology ranges from transient viral infections and asthma to rare congenital and structural airway abnormalities or genetic disorders. Accurate differentiation is crucial for prompt management, especially in children under three years of age.
Aims: To determine the spectrum and frequency of wheezing causes in toddlers and identify clinical red flags that necessitate a targeted diagnostic work-up.
Methods: A cross-sectional study included 60 toddlers with wheezing. Baseline assessment for all participants consisted of clinical history, physical examination, and blood tests. Targeted second-line investigations were performed based on clinical indications: radiological and functional studies (chest X-ray, computed tomography, ultrasonography, bronchoscopy, echocardiography). Final diagnoses were categorized as asthma, acute respiratory infections, cystic fibrosis, vascular ring, hemangioma, and other causes. Descriptive statistics were used to assess frequencies and proportions.
Results: Among 60 children, bronchial asthma was the most frequent etiology – 32 cases (53.3%). Respiratory infections accounted for 12 cases (20.0%). Rare pathologies – specifically cystic fibrosis, vascular ring, and hemangioma – were identified in one child with each of these pathologies (1.7% per condition). Other causes – including transient or idiopathic wheezing and nonspecific bronchial hyperreactivity – accounted for 13 cases (21.7%). The majority (75%) of asthma-related wheezing cases had recurrent episodes, whereas rare etiologies such as cystic fibrosis and vascular anomalies were associated with persistent symptoms from early infancy, failure to thrive and poor response to bronchodilator therapy.
Conclusion: In this cohort, more than half of wheezing cases in children under three years old were related to bronchial asthma, whereas approximately 5% were due to rare structural or genetic disorders. Targeted investigations (sweat test, echocardiography, CT/bronchoscopy) are recommended selectively when atypical features – such as onset in early infancy, growth failure, or poor response to standard therapy – are present. This approach ensures early identification of serious underlying conditions while avoiding unnecessary medical interventions. The proposed diagnostic approach may improve early identification of serious underlying conditions.

Funding: The study was supported by the State Non-Profit Enterprise “Danylo Halytsky Lviv National Medical University”.
Ethical Committee Approval: Nonprofit Communal Enterprise “City Children’s Clinical Hospital of Lviv”; November 16, 2021 № 12.
Acknowledgement: We thank Cedars-Sinai Medical Center’s International Research and Innovation in Medicine Program, the Regional Cooperation for Health, Science and Technology Association (RECOOP HST Association) for their support of our organization as participating Cedars-Sinai Medical Center - RECOOP Research Center (CRRC).

RECOOP Publications
2025



Juwel bug

Yevhenii Kucheriavyi
Palladin Institute of Biochemistry NAS of Ukraine, Kyiv, Ukraine

RECOOP publications in 2025

Within the framework of the International Research and Innovation in Medicine (IRIM) Program at Cedars-Sinai Medical Center (CSMC), Sandor G. Vari organized the Regional Cooperation for Health, Science, and Technology (RECOOP HST) Association. The Association's primary goal is to enhance research collaboration and provide platforms for scientific networking in the life sciences within the Central and Eastern European (CEE) Countries and the Cross-Atlantic. I resigned as President of the Association, and now I serve as Chairman of the Scientific Advisory Board. In 2014, I implemented a competitive grant system in the RECOOP HST Association. I managed several RECOOP Grants and implemented 38 projects. The real measure of the scientific outcome is the quality of published papers. It is my last action in RECOOP to close out the projects I started with the RECOOP members. The real measure of the scientific outcome is the quality of published papers. We are publishing our results in Q1 (Quartile 1) and Q2 (Quartile 2) journals. RECOOP papers are top-tier academic publications ranked by citation impact and prestige within their subject category, usually indexed in Scopus (via SJR) or Web of Science (via JCR). Q1 journals represent the top 25% (highest impact), while Q2 journals fall in the 25–50% range.

In 2025-26, I successfully closed out numerous RECOOP–CSMC Grants and projects and authored **12** publications with an IF 47,5.

Kolobarić, Nikolina, Zrinka Mihaljević, Mirjana Suver Stević, Ana Marinčić Žagar, Sandor G. Vari, and Ines Drenjančević. "Polyunsaturated Fatty Acid (PUFA) Composition of Growth Medium Changes the Atherogenic Potential of Human Aortic Endothelial Cells (HAECs) Following Endotoxin Stimulation" *Biomedicines* 13, no. 11: 2706.
https://doi.org/10.3390/biomedicines13112706 *Impact Factor 3.9*
RECOOP Bohdan Malaniak Young Scientists Research Grant #33 Polyunsaturated fatty acid composition

Yatsenko, Tetiana, Iryna Us, Daria Korolova, Svitlana Zhuk, Halyna Dziuba, Alona Nalbat, Svitlana Kharchenko, Sandor George Vari, and Volodymyr Chernyshenko. "Placental Dysfunction Is Associated with Dysregulated Fibrinolytic System Activation." *International Journal of Molecular Sciences* 26, no. 19 (2025): 9339. https://doi.org/10.3390/ijms26199339 *Impact Factor of 4.9*
Cedars–RECOOP Solidarity Fellowship (provided by RECOOP HST Association, CRSF #1

Korolova, D.; Suranyi, A.; Pavlenko, A.; Altorjay, A.T.; Zhuk, S.; Us, I.; Melnyk, Y.; Chernyshenko, V.; Vari, S.G. Obesity Is a Thrombotic Risk Factor in Pregnant Women. J. Clin. Med. 2025,14,5310. https://doi.org/ 10.3390/jcm1415531 *Impact Factor 3.3*
Cedars–RECOOP Solidarity Fellowship CRSF # 1

Farkas, Anna, Oksana Matsyura, Lesya Besh, Andras Guttman, and Sandor GG Vari. "IgG N-Glycan Profiles in Mothers and Infants Postpartum in the Context of Maternal Obesity and Gestational Diabetes." International Journal of Molecular Sciences (2025).
https://doi.org/10.3390/ijms262110641 *Impact Factor of 4.9*
RECOOP HST Association, Fusion Grant number # 016: 2019 – 2021. Extension 2022 Vass-Ertl. Pecs: Pregnant Obesity and GDM changing human milk secretory

Kozak, Kateryna, Halyna Pavlyshyn, Oleksandr Kamyshnyi, Oksana Shevchuk, Mykhaylo Korda, and Sandor G. Vari. "The influence of genetic polymorphisms on cytokine profiles in pediatric COVID-19: a pilot study." *Frontiers in Pediatrics* 13 (2025): 1523627. https://doi.org/10.3389/fped.2025.1523627 *Impact Factor* ***2.1***
RECOOP HST Association Fusion Grant # 030 COVID-19 Severity and Gene Polymorphism in Children and Adults

Kozak, Kateryna, Halyna Pavlyshyn, and Sandor George Vari. "Prognostic Value of Caspase-3 and Cardiac Troponin I in Assessing Cardiovascular Risk in Pediatric COVID-19 and Multisystem Inflammatory Syndrome." *Journal of Multidisciplinary Healthcare* (2025): 3009-3019. https://doi.org/10.2147/JMDH.S514776 *Impact Factor 4.5*
RECOOP HST Association Fusion Grant # 030, COVID-19 Severity and Gene Polymorphism in Children and Adults

Farkas, Anna, Andrea Suranyi, Balint Kolcsar, Zita Gyurkovits, Zoltan Kozinszky, Sandor G. Vari, and Andras Guttman. "Potential Glycobiomarkers in Maternal Obesity and Gestational Diabetes During Human Pregnancy." *Journal of Clinical Medicine* 14, no. 5 (2025): 1626. https://doi.org/10.3390/jcm14051626 *Impact Factor: 3.3*
RECOOP Young Scientists Research Grant BMYS #008 2019–2020 Farkas—Guttman

Ivić, Vedrana, Irena Labak, Oksana Shevchuk, Rudolf Scitovski, Viktoria Ivankiv, Kateryna Kozak, Mykhaylo Korda, Marija Heffer, and Sandor G. Vari. "Sex-Specific Patterns of Cortisol Fluctuation, Stress, and Academic Success in Quarantined Foreign Medical Students During the COVID-19 Lockdown." *Life* 16, no. 1 (2025): 54. Journal Impact https://doi.org/10.3390/life16010054 *Impact Factor of* ***3.4***
RECOOP–Cedars-Sinai Senior Scientists Grant #021 2020 2021 Sex difference in chronic stress during the COVID-19 lockdown of students.

Kramar, Solomiia, Zoia Nebesna, Yuliia Yakymchuk, Alla Boychuk, Oksana Shevchuk, Mykhaylo Korda, and Sandor George Vari. "Changes in Placentas of Pregnant Women Infected with COVID-19." *International Journal of Molecular Sciences* 26, no. 17 (2025): 8596 https://doi.org/10.3390/ijms26178596. *Impact Factor of 4.9*
RECOOP Grant# 028 Fusion Grant 2020–2021 COVID-19 disease and comorbidities during pregnancy

Vass RA, Miko E, Premusz V, Vari SG, Kovacs K, Bodis J, Ertl T. The Placenta-Gut Microbiota Axis in Gestational Diabetes Mellitus: Molecular Mechanisms, Crosstalk, and Therapeutic Perspectives. Int J Mol Sci. 2025 Dec 27;27(1):312. *Impact Factor of 4.9*
doi: 10.3390/ijms27010312
RECOOP HST Association, Fusion Grant number # 016: 2019 – 2021. Extension 2022 Vass-Ertl. Pecs: Pregnant Obesity and GDM changing human milk secretory

Hrebenyk, M., Maslii, S., Shevchuk, O., Komorovsky, R., & Korda, M. (2025). Impact of Hypertension and Antihypertensive Treatment on COVID-19 Severity: A Retrospective Observational Study in Ternopil Region, Ukraine. Therapeutics and Clinical Risk Management, 21, 995–1007. https://doi.org/10.2147/TCRM.S527151 Impact *Factor* 2.5
RECOOP research grant #023 RCSS 2020-2021, project focused on the impact of COVID-19 infection on patients with high blood pressure, specifically those using ACEI and ARB medications. I created and helped to implement the project. Since the Ternopil team did not

give me credit for my idea and work, and I was not the senior author of this paper, I requested that my name not be included as an author.
Reference to
Shevchuk O, Pak A, Palii S, Ivankiv Y, Kozak K, Korda M, Vari SG. Blood ACE2 Protein Level Correlates with COVID-19 Severity. Int J Mol Sci. 2023 Sep 11;24(18):13957. doi: 10.3390/ijms241813957. *Impact Factor of 4.9*
RECOOP Research Grant #023 "Impacts of COVID-19 infection on patients with high blood pressure using angiotensin-converting enzyme inhibitors (ACEI), angiotensin-receptor blockers (ARBs)", the RECOOP Research Grant #024 "Vascular Dysfunction in post-infected COVID-19 patients.

Under preparation
Exploring Serum IgA N-Glycosylation Changes in Gestational Diabetes Mellitus
Anna Farkas, Oksana Matsyura, Lesya Besh, Rebeka Torok, Andras Guttman, and Sandor G. Vari. Submitted to Int J Mol Sci. 2025 Dec 27;27(1):312. *Impact Factor of 4.9*
RECOOP HST Association, grant number 'FUS 016: 2019–2021 extension 2022 Vass-Ertl. Pecs': Pregnant Obesity and GDM changing human milk secretory cytokines, and altering IgG-IgA N-glycans and fatty acids

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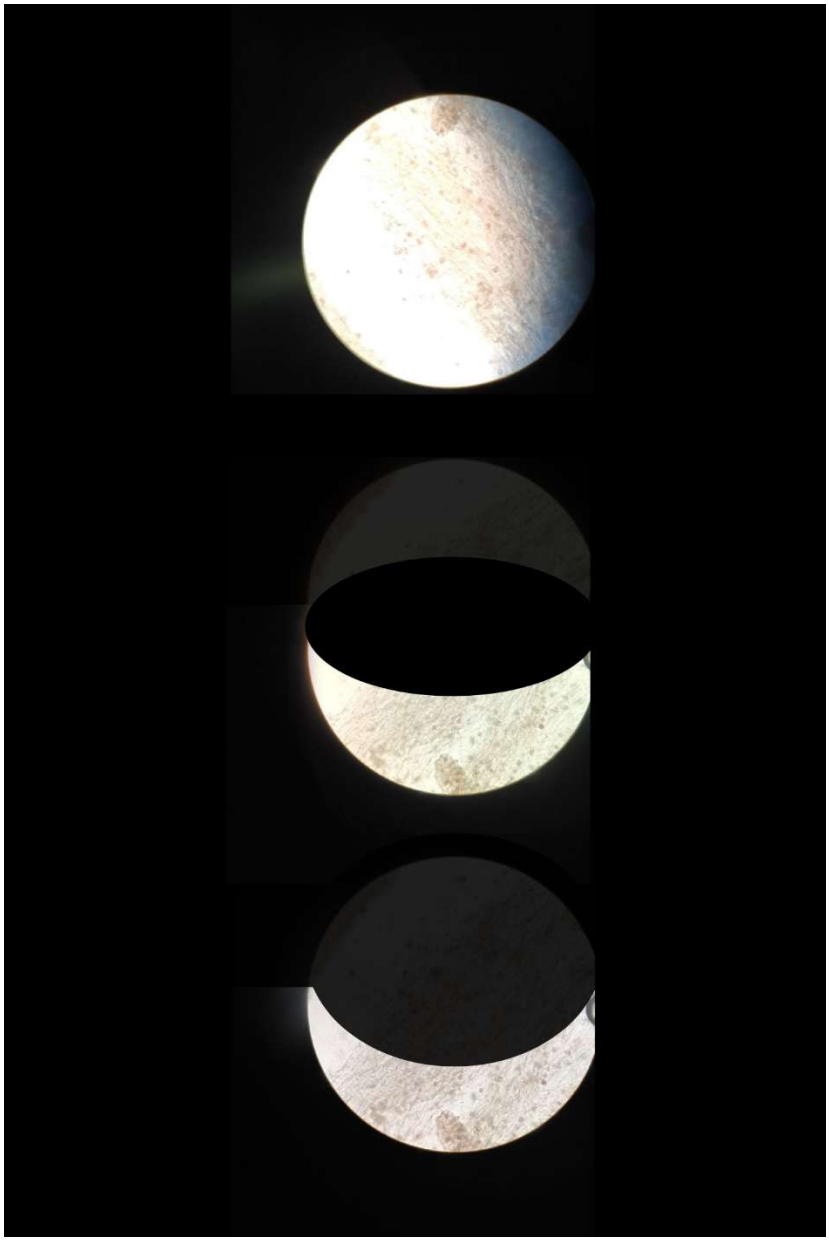

Phases of Mushrooms Moon

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