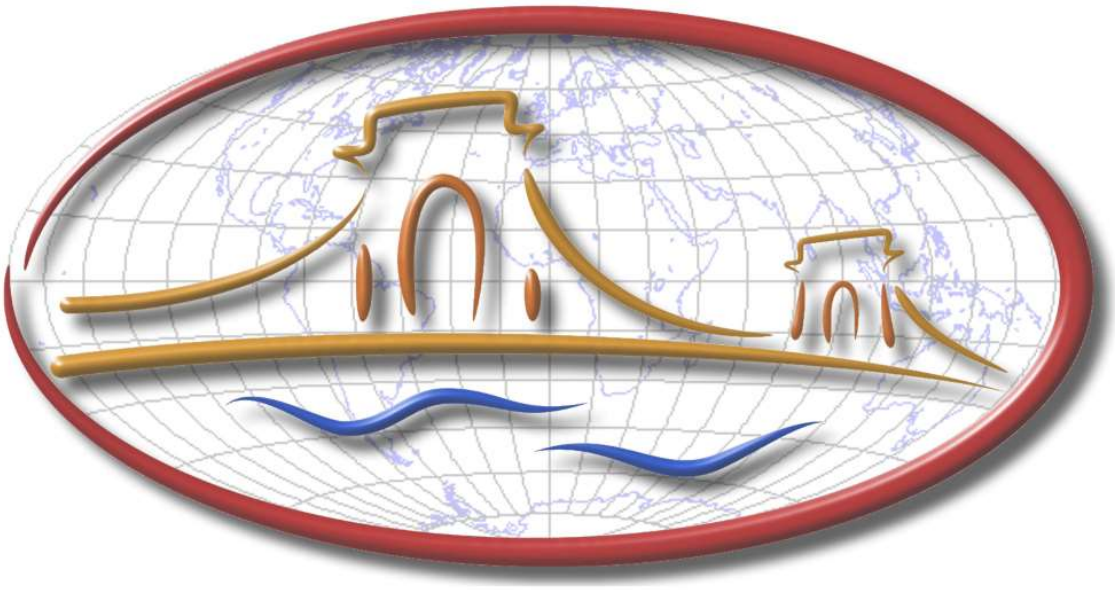

RECOOP HST ASSOCIATION

Bridges in Life Sciences

RECOOP 13th Annual Scientific Conference

Hotel International

Zagreb, Croatia

Organizing Committee

USA

Edward Prunchunas
Cedars-Sinai Medical Center & RECOOP HST Association

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Scientific Advisory Board

Preface

In 2006, Cedars–Sinai Medical Center (CSMC) with eleven Central and Eastern European (CEE) universities and academic organizations from six countries (Croatia, Czech Republic, Hungary, Romania, Slovakia, and Ukraine) formed the Regional Cooperation for Health, Science and Technology (RECOOP HST) Consortium.

In 2012, CSMC and the CEE partner organizations agreed to form the Association for Regional Cooperation in the Fields of Health, Science and Technology (RECOOP HST Association https://www.cedars-sinai.edu/Research/Research-Administration/Recoop/).

RECOOP is a strategic partnership that provides added value to both CSMC and the CEE organizations. RECOOP organizes meetings and conferences that help the participating scientists to understand the most important element of knowledge sharing: within collaborative research each member depends on the group and building of trust within the group is the key to success. At the same time however, it is necessary to empower the participating scientist to be creative and autonomous.

In RECOOP, science enables researchers to be creative and motivate researchers to acquire knowledge needed by every university graduate, PhD student and postdoc. It is also useful in the selection of future researchers and teachers. Science is not perfect and sometimes produces controversial results, but at the same time, it teaches analytical thinking skills that are very valuable in modern societies.
Vari S. G. Knowledge sharing is the key for the progress of science. The Ukrainian Biochemical Journal 2018; Volume 90, N 2, 5-7

Pre – Conference Workshop
April 12, 2018

The pre-conference Workshop is part of the RECOOP 13th Bridges in Life Science Annual Conference on April 12 – 15, 2018 at the Hotel International Zagreb, Miramarska 24, Zagreb 10000, Croatia http://www.hotel-international.hr

Registration desk is at the Corridor of the Conference Rooms from 07:30 am to 07:00 pm.

Knowledge Transfer Pathways from Research to Patient Treatment

Adriatic Room

Science – Knowledge Transfer - Innovation Management - Clinical Investigation

Keywords: develop, aware, protect, accept, investigate, demonstrate, agree, benefit

Develop - scientists develop new methods, markers, tests, instruments
Aware – scientists realize the scientific value of their research and apply for protection of their research output
Protect – technology transfer professionals initiate Intellectual Property Protection
Accept – publish results and the scientific community accepts the novelty of their approach
Investigate– conduct market research and investigate the market value for medical use
Demonstrate – initiate clinical research to demonstrate the invented methods, markers, tests, or instruments are able to perform the predicted, but not harmful, well-defined medical use
Agree – the medical community accepts, and the interested companies realize the market opportunities
Cost-Benefit - investigate the cost-benefit features of the new application for medical practice

Workshop Agenda

08:30 – 08:45 Welcome – Dr. Sandor G. Vari

Knowledge Transfer Chain Reaction ™
Develop - Aware - Protect - Accept – Investigate - Demonstrate – Agree – Benefit

08:45 – 09:00 Introduction of the Faculty
Sandor G. Vari

09:00 - 09:45 Develop - scientists develop new methods, markers, tests, instruments **Q&A**
Sandor G. Vari
Most scientists are self-directed and they desire to make discoveries independently. In the 21st Century, this situation is changing rapidly and nowadays researchers and research institutions look for collaborations to enable capacity building and offer multiple opportunities that ~~to~~ surpass the limitations of a single institution and scarce resources. The ongoing process of knowledge integration is removing territorial borders and fostering research collaborations to expedite laboratory research. The molecular information gained through various laboratory techniques is directly used to develop new ways to treat patients.

09:45 – 10:30 Aware – scientists realize the scientific value of their research and apply for protection of their research output **Q&A**
Tamas Bene
Researchers need to be aware of the IP protection potential of their research results to avoid premature publication, since it can jeopardize protection and eventually it can hinder the whole technology transfer process. In this presentation, the various forms of IP protection will be discussed, with special attention to patenting, copyright and know-how. The section will provide a detailed overview of the patent prosecution process and we will discuss how to resolve the conflict between publishing and patenting.

10:30 – 11:00 Coffee Break

11:00 – 11:45 Protect – technology transfer professionals initiate Intellectual Property Protection **Q&A**
Tamas Bene
Technology transfer describes the process of formally transforming research results into practical applications with commercial potential, seeking intellectual property protection for these innovations, and then transferring them to industry via license agreements or sometimes spin-off companies. Even at the earliest stage of academic research, it is essential that researchers understand the basics of intellectual property (IP) management in order to maximize the economic benefit from public research and to be able to develop new and marketable products and services. The session will introduce the most important aspects of university IP management.

11:45 – 13:00 Accept – publish results and the scientific community accepts the novelty of their approach **Q&A**
Srecko Gajovic
"Publish or perish" evolved to become the mantra of the scientific community. Hence, the number of publications increased immensely and their importance for individual careers, promotions, grants and research status became paramount. Subsequently, the early-career stage researchers should be careful from the very beginning of their careers if their research efforts would be publishable. This consideration implies planning the hypothetical publication from the first steps, and building collaborations with justifiable contributions from co-authors for the envisaged research papers.

13:00 – 14:00 Lunch Break

14:45 – 15:30 Investigate– conduct market research and investigate the market value for medical use **Q&A**
Nirdesh K. Gupta
In this session, we will discuss how to understand the potential value of your inventions, best practices to conduct market research and identify the industry landscape, as well as how to best position your technology by showcasing competitive advantages. We will cover resources and tools for conducting market analysis and identifying prospective licensees. Furthermore, we will also share our thoughts on how to develop marketing strategy, write effective technology summaries, and market the technology to the appropriate industry contact to assess licensing interest.

15:30 – 16:15 Demonstrate – initiate clinical research to demonstrate the invented methods, markers, tests, or instruments are able to perform the predicted, but not harmful, well- defined medical use **Q&A**
Linn Defensor
This section of the workshop will provide an overview of the proper conduct of clinical research: interactions and relationships between basic research and clinical research, the different kinds of clinical research, the activities involved during clinical research implementation and regulations and guidelines that govern the conduct of clinical research. This section will define the members of the research team and their responsibilities. It will discuss the biggest revision to ICH-GCP in 20 years which became effective in June 2017. It will touch on an important aspect of research called quality management or QM. QM activities from before the start of the study and during the conduct of the study will be described. Common errors that occur during the different stages of clinical research will be defined.

16:30 – 17:00 Coffee Break

17:00 – 17:45 Agree – the medical community accepts, and the interested companies realize the market opportunities **Q&A**
Nirdesh K. Gupta
This session discusses the art and skills of negotiation – an essential component to bring the licensing transaction to completion. Dealmaking is not a zero-sum game. We will share experiences on how to keep your goal in mind, strategically structure your deal to gain the maximum value, and reach agreements in which both sides of the table are content - all the while building a long-lasting relationship with your licensee!

17:45 – 18:30 Cost-Benefit - investigate the cost-benefit features of the new application for medical practice **Q&A**
Norbert Buzas
Cost-benefit analysis is an important part of decision making in the application of new products and technologies. However, since the goal of healthcare is restoring human health, an aspect that cannot be expressed in money, the classic cost-benefit analysis in healthcare is expanded by adding a moral dimension. The discussion in this workshop section will demonstrate how to make the cost-benefit analysis of healthcare technologies still possible, how cost-benefit comparisons can be made for certain therapies and what unexpected costs may be incurred if the new technologies are misused.

18:30 – 19:00 Interactive Discussion
Moderator: Sandor G. Vari

20:00 – 22:00 Dinner - Restaurant and Grand Salon II

Agenda of RECOOP 13th Bridges in Life Sciences Annual Conference
April 13, 2018

08:30 am - 09:00 am **Welcome and Review of RECOOP Activities**
Adriatic Room
Sandor G. Vari

09:00 am – 09:40 am **RECOOP-CSMC Senior Scientist Award Competition**
Adriatic Room
10 minutes oral presentations

Session Chairs
Sandor G. Vari
Tibor Ertl
Ines Drenjancevic
Srecko Gajovic
Tamas Tabi

The role of obesity-induced low-grade inflammation in adipokine signaling in the pregnant rat uterus
Robert Gaspar

Deciphering the role of NET formation in NASH pathogenesis and testing of potential preventive drug candidates.
Rostyslav Bilyy

Obesity-induced cooperation of BMP and Wnt signaling pathways in the mediation of uterine myometrium apoptosis
Olexandr Korchynskyi

09:30 am – 09:40 am **Q&A + Discussion**

09:40 am - 11:00 am **Plenary Session - RECOOP Research in Progress (RRP)**
Part I
Adriatic Room
10 minutes oral presentations

Session Chairs
Tatiana Borisova
Elena Pieckova
Eva Szoko
Robert Gaspar
Charles F. Simmons

Connexin 37 gene polymorphism and atherosclerotic changes in diabetic women type 1 and
Jan Pitha

Influence of Different Diets on Fatty Acid Composition in Plasma of Rats
Geza Muranszky

Correlation between placental volume measured by ultrasound in-vivo, and sFlt-1/PlGF ratio in preeclampsia
Abel T. Altorjay

Medicinal chemistry of 4-thiazolidinones: New challenges and solutions
Roman Lesyk

Small but smart actors in big performance - the nanomedicines
Rostyslav Stoika

Imaging parameters of repair after ischemic brain lesion in the mouse
Srecko Gajovic

Conjugation of 4-thiazolidinone derivatives with PEGylated nanocarrier enhances their treatment effect, improves water solubility, and reduces general toxicity in tumor-bearing mice
Lesya Kobylinska

11:00 am – 11:15 am **Q & A + Panel Discussion**

11:15 am – 11:30 am **Coffee Break - Conference Section Lobby**

11:30 am – 12:00 pm **Opening Ceremony of Sciences and Arts Exhibit**
Mediteran Room
Csaba Valdar
Kenneth Anthony Flewellyn
Toni Franovic

12:00 pm – 01:00 pm **Plenary Session - RECOOP Research in Progress (RRP) Interactive Poster Presentations *Part I***
Adriatic Room
5 minutes oral presentations

Session Chairs:
Ines Drenjancevic
Lesya Kobylinska
Jan Pitha
Rostyslav Stoika
Roman Lesyk
Srecko Gajovic

1., Enhancing the effectiveness of low-level laser radiation with nanoparticles on clinical strains of Staphylococcus aureus
Marta Panas

2., Search for New Anticancer Agents Among Thiazole Derivatives With Michael Acceptor Functionality
Danylo Kaminskyy

3., Enhancement of water solubility and innate MDR-circumventing activity of landomycin A by its immobilization on poly(2-oxazoline) nanocarrier
Rostyslav Panchuk

4., 4-Thiazolidinone derivatives change the membrane potential of rat brain nerve terminals
Marina Dudarenko

5., pH dependent fluorescence of CdSe/CdS nanoparticles in an aqueous phase
Anna Lesiak

6., Polymer-coated upconversion and magnetic nanoparticles for biomedical applications
Uliana Kostiv

7., Semiconductor lead sulfide nanocrystals for infrared bio-medical imaging
Hanna Woznica

8. Antioxidant core-shell iron oxide nanoparticles and their cellular interactions
Maksym Moskvin

9., Neurotoxic level of synthesized, native and physiological nanoparticles: a comparative analysis
Arsenii Borysov

10., Assessment of accumulation of carbon nanodots in rat tissues after intravenous administration
Maksym Galkin

01:00 pm – 02:00 pm **Interactive Poster Part I Q&A + Discussion at the Posters**
(It will be at the assigned poster number, size W 90 cm & H 120 cm)
Grand Salon I
Ines Drenjancevic
Lesya Kobylinska
Jan Pitha
Rostyslav Stoika
Roman Lesyk
Srecko Gajovic

01:00 pm – 02:00 pm **Sciences and Arts Exhibit - Mediteran Room**

02:00 pm – 03:00 pm **Lunch Break - Restaurant and Grand Salon II**

03:00 pm – 05:00 pm **Sciences and Arts Exhibit - Mediteran Room**

03:00 pm – 03:30 pm **Twin Registry Brainstorming** - *Adriatic Room*
Moderators: Sandor G. Vari, Adam Tarnoki, and David Tarnoki

Invited
Reproductive Health Research Platform: Tibor Ertl, Charles Simmons, Andrea Suranyi, Robert Gaspar.

Osijek: Ines Drenjancevic, Marija Heffer, Andrijana Muller, Senka Blazetic, Marta Balog
Zagreb: Srecko Gajovic, Anton Glasnovic, Smiljka Vikic, Dora Visnjic
IKEM: Jan Pitha, Irena Markova
SMU: Elena Pieckova; Brigita Benkoova; Michaela Pospisilova
DHLNMU: Lesya Kobylinska, Oksana Matsyura, Andrii Lozynskyi, Dmytro Besh, Sviatoslav Fetko, Marta Panas, Maria Sorochka
ICB: Rostyslav Stoika; Rostyslav Panchuk; Julia Kozak; Nataliya Finiuk

03:30 pm – 05:00 pm **RECOOP 10th General Assembly**
Adriatic Room

05:00 pm – 06:00 pm **Discussion of the NanoBioTechnology Book for Springer, Biomedical Engineering**
Adriatic Room

Chapters

Chapters
New trends in nanobiotechnology
Sandor G. Vari

Nanoparticles in Biological Imaging
Srecko Gajovic

Biological applications of nanocrystals
Artur Podhorodecki – Rostyslav Bilyy

Role of Magnetic Iron Nanoparticles in Life Sciences and Medical Imaging
Daniel Horak

Controlled drug delivery and reduced side effects of cancer drugs with nanocarriers
Rostyslav Stoika, Roman Lesyk, Rostyslav Panchuk and Lesya Kobylinska

Environmental impact of nanoparticles
Tatiana Borisova and Sandor G. Vari

Enjoy the city on your own!


GORNJI GRAD
KAPTOL
Upper Town
ŠALATA
DONJI GRAD
Lower Town

RECOOP 13th Bridges in Life Sciences Annual Conference
April 14, 2018

08:00 am - 08:20 am **Report the outcomes of General Assembly**
Adriatic Room
Sandor G. Vari

08:20 am - 09:20 am **Plenary Session – Excellence in Students Research**
Adriatic Room
10 minutes oral presentations

Session Chairs
Marija Heffer
Andras Guttman
Sandor G. Vari

Nimodipine treatment preserves the efficacy of neurovascular coupling in the ischemic rat cerebral cortex
Dora Hantosi

Assessment of the small intestinal blood flow by indocyanine green fluorescence using color-fluorescence laparoscope
Shohei Yoshida

Nanofabricated poly(vinyl alcohol) scaffolds for abdominal hernia repair
Constantinos Voniatis

The rapid effect of 17-β-estradiol on diffusion dynamics of p75 receptor in live neurons
Andras Straub

Sexually differences in the biomechanics and vasoreactivity of coronary resistance arteries in exercise induced left ventricular hypertrophy
Eszter Horvath

09:20 am - 09:30 am **Q&A + Panel Discussion**

09:30 am – 10:50 am **Plenary Session – Life Sciences Progress in Ukraine reported in The Ukrainian Biochemical Journal**
http://ukrbiochemjournal.org/
Adriatic Room
10 minutes oral presentations

Session Chairs
Rostyslav Stoika
Roman Lesyk
Tatiana Borisova

Blood coagulation and aortic wall integrity in rats with obesity-induced insulin resistance
Oksana Dziuba

Clot formation and lysis in platelet rich plasma of healthy donors and hypertensive patients
Olga Revka

Vitamin D3 improves bones biomechanical properties through NF-κB - VDR-related pathway in diabetes
Tatiana Borisova and Volodymyr Chernyshenko

Evaluation of antiproliferative activity of pyrazolotriazolopyrimidine derivatives
Nataliya Finiuk

The impact of N-Acetylcysteine on antitumor activity of Doxorubicin and Landomycin A in Nk/Ly lymphoma-bearing mice
Julia Kozak

Research and development of peritoneal dialysis solutions
Nataliia Hudz

Simple two-step covalent protein conjugation to PEG-coated nanocrystals
Rostyslav Bilyy

10:50 am - 11:00 am **Q&A + Panel Discussion**

11:00 am - 11:30 am **Coffee Break with Scientist and Artists**
Sciences and Arts Exhibit
Auction of the Life Science Images and Artworks
Mediteran Room and Conference Section Lobby

11:30 am – 12:40 pm **Plenary Session RECOOP Research in Progress (RRP)**
Interactive Poster Presentations *Part II*
Adriatic Room
5 minutes oral presentations

Session Chairs
Senka Blazetic
Marija Heffer
Andrijana Muller
Buzas Norbert
Daniel Horak
Volodymyr Chernyshenko

11., Sex differences of metformin effects on metabolic risk factors in patients with metabolic syndrome.
Maria Sorochka

12., The expression of the connexin 37 gene in the aorta of rat models of dyslipidemia, hypertension and dicarbonyl stress
Irena Markova

13., Ultrasonographic aspect of glycemic control of placental vascularization in type 1 diabetes mellitus
Andrea Suranyi

14., Might Fungal Sinusitis Be Related to Lowered Indoor Air Quality?

Elena Pieckova

15., Gender difference in glucocorticoid, insulin and estrogen receptors expression upon chronic stress and aging
Marta Balog

16., The common effect of early transient hyperglycaemia and hyperoxia on the retina and kidney
Dorottya Balika

17., Investigation of prenatal smoke-exposure in chronic retinal hypoperfusion on adult rats
Barbara Mammel

18., Effects of Olaparib and radiation on cervical cancer cell line
Reka Anna Vass

19., Comparative pharmacokinetic study of levocetirizine in pregnant and non-pregnant rats
Anita Sztojkov-Ivanov

20., Changes in the cervical resistance by COX-inhibitors and alpha-tocopherol in rat
Anna Kothencz

21., Improvement of virtual screening efficacy with a property-based scoring scheme for finding glucocorticoid receptor modulators
Ibolya Toth

22., Study of Viral Kinetics and Histopathology of CVB3 infection in C57BL/6 male mice
Brigita Benkoova

23., Relationships among different adherences of type 2 diabetic patients
Andrea Klinovszky

24., Anticoagulant action of fibrinogen-specific proteases in vitro
Ievhenii Stohnii

12:40 pm – 01:00 pm	**Interactive Poster *Part II* Q&A + Discussion** ***(It will be at the assigned poster number, size W 90 cm & H 120 cm)*** *Grand Salon I* Senka Blazetic Marija Heffer Andrijana Muller Buzas Norbert Daniel Horak Volodymyr Chernyshenko
12:40 pm – 01:00 pm	**Mingling Scientist and Artists - Sciences and Arts Exhibit** *Mediteran Room*
01:00 pm – 02:00 pm	**Lunch Break - Restaurant and Grand Salon II**

02:00 pm – 04:00 pm **Plenary Session RECOOP Research in Progress (RRP) Part II**
&
RECOOP Young Scietists Research Grants
Adriatic Room
10 minutes oral presentation

Session Chairs
Tatiana Borisova
Elena Pieckova
Eva Szoko
Tibor Ertl
Charles F. Simmons

Characterization of blood coagulation system of morbidly obese patients
Volodymyr Chernyshenko

Capillary electrophoresis as a molecular diagnostics discovery tool for common mechanisms of diseases
Andras Guttman

Body Mass Index and Markers for Chronic Inflammatory Response: High Sensitive C-Reactive Protein and Procalcitonin in the Third Trimester of Pregnancy
Andrijana Muller

Anti-inflammatory effects of the omega-3 fatty acids
Ines Drenjancevic

Changes in genetic and environmental influences on arteriosclerotic traits: a longitudinal twin study
Adam D Tarnoki

The mechanism of 5-aminoimidazole-4-carboxamide ribonucleoside-mediated antiproliferative and differentiative effects on acute myeloid leukemia cells
Dora Visnjic

Association of mtDNA copy number and telomere length with lumbar disc degeneration: a twin study
David L Tarnoki

BOHDAN MALANIAK Young Scientist Award Competition
Session Chairs
Sandor G. Vari
Tibor Ertl
Ines Drenjancevic
Srecko Gajovic
Tamas Tabi

Protein biocorona formation at the nanoparticle surface and consequent changes in their properties during medical application
Arsenii Borysov

Correlation between placental vascularization indices, sFlt-1/PlGF ratio and blood coagulation factors in pregnancy hypertension.
Abel Tamas Altorjay

BOHDAN MALANIAK Young Scientist Award Progress Report

Polymorphic variants of interleukin-13 R130Q and interleukin-4 T589C in children with and without cow's milk allergy
Oksana Matsyura

Superparamagnetic iron oxide nanoparticles as bio-imaging agent and carrier of anticancer drugs.
Andrii Lozynskyi

03:50 pm – 04:00 pm	**RECOOP Research Grants and Research in Progress Q&A + Panel Discussion**
04:00 pm - 04:30 am	**Coffee Break with Scientist and Artists** **Closing Remarks and Announcement of the Sciences and Arts Exhibit Winners - Mediteran Room and Conference Section Lobby** Csaba Valdar Kenneth Anthony Flewellyn Toni Franovic
4:30 pm – 06:15 pm	**RECOOP Research in Progress – Interactive Poster Session *Part III*** *Adriatic Room* 5 minutes oral presentations

Session Chairs
Tatiana Borisova
Andrea Suranyi
Rostyslav Bilyy
Srecko Gajovic
Charles F. Simmons

25., Evaluating scientific excellence in relation to received international grants
Olja Ulicni Niksic

26., Activities of Research and Technology Transfer Office of the University of Zagreb School of Medicine
Smiljka Vikic Topic

27., Retracted papers of the Croatian authors in the international bibliographic databases
Anton Glasnovic

28., Relation between the antiquity of intracoronary thrombi and prognosis of patients with acute ST segment elevation myocardial infarction
Dmytro Besh

29., The influence of atorvastatin on omentin level and HOMA-IR in patients with coronary artery disease and obesity
Tetiana Maksymets

30., Antiviral activity of sophorolipids against MHV-68
Michaela Pospisilova

31., Genetic versus environmental effects on the relationship between lumbar degeneration and low back pain: results of an MR twin study
Andras Dienes

32., Association between obstructive sleep apnea and lumbar disc degeneration: evidence of a genetic link
Marcell Szily

33., Size of the pituitary gland and its connection with body mass index: genetics or environment?
Aliz Persely

34., Research and development of gingival gel on the base of ornidazole, chlorhexidine bigluconate, xylitol and essential oils
Galyna Demchyna

35., The role of component resolved diagnostics in treatment and prevention of food anaphylaxis in pediatric practice
Oksana Matsyura

36., The ways for improving the bioavailability among biologically active 4-thiazolidinones and their structure-related analogs
Andrii Lozynskyi

37., Beekeeping products as active substances of medicinal products
Oksana Yezerska

38., Application of flexible nanoholey patches for healing of infected skin wounds
Solomiya Paryzhak

39., Better Survival and Delayed Neuronal Stress After Stroke in Mice Lacking TLR2
Dunja Gorup

40., Assessment of Preparation Protocols for Multimodal ex vivo Mouse Ischemic Brain Lesion Visualization
Helena Justic

41., Activation of neutrophil extracellular traps under high-fat high-cholesterol diet
Tetiana Dumych

42., Obesity-provoked changes in bone morphogenetic protein and inhibitor of DNA-binding: levels in placenta of pregnant rats and blood plasma of pups
Nataliya Finiuk, Olexandr Korchynskyi

43., Cytotoxic Effect of Recombinant Analog of Lactaptin Rl2 is Associated with Enhanced ROS Production in Tumor Cells
Nazar Manko

06:15 pm – 07:00 pm	**RECOOP Research in Progress (RRP) Interactive *Poster Part II*** **Q&A + Discussion** ***(It will be at the assigned poster number, size W 90 cm & H 120 cm)*** *Grand Salon I* Senka Blazetic Marija Heffer Andrijana Muller Daniel Horak Volodymyr Chernyshenko **RECOOP Research in Progress (RRP) Interactive Poster *Part III*** **Q&A + Discussion** ***(It will be at the assigned poster number, size W 90 cm & H 120 cm)*** *Grand Salon I* Tatiana Borisova Andrea Suranyi Rostyslav Bilyy Srecko Gajovic Charles F. Simmons **Auction of the Life Science Images and Artworks** *Mediteran Room*
07:00 pm – 07:30 pm	**Closing Remarks**
08:00 pm	**Dinner - Restaurant and Grand Salon II**

April 15, 2018
Departure

RECOOP next meetings

RECOOP 9th Annual Project Review – TriNet Meeting on October 12 – 13, 2018, arrival on October 11 (Thursday) and departure on October 14, (Sunday) 2018
Hotel TATRA, a.s.
web: www.hoteltatra.sk

RECOOP 14th Bridges in Life Science Annual Conference on April 12-13, 2019 on arrival April 11 (Thursday) and departure on April 14, (Sunday) 2019
Hotel TATRA, a.s.

NanoBioTechnology Book for Springer, Biomedical Engineering

New trends nanobiotechnology Sandor G. Vari

Clinical treatment of patients targets health at the individual level, whereas the objectives of public health are to promote, protect, and preserve health for groups of people or populations. It is essential to investigate and explore the potential applications and impacts of nanomedicine in clinical medicine and for public health. Nanotechnology is growing rapidly in the biomedical field, where advances are being made in both diagnostics and treatment areas. In the diagnostics area, nanosensors that can detect, identify, and quantify biological substances in body fluids are leading to early disease detection and earlier treatments as well as the ability to detect environmental contaminants in the blood. A life-threatening condition that can arise from an infection caused by bacteria, fungi, or viruses—impacts millions of people each year. Nanotechnology could detect infection from a blood sample and determine which drugs will be most effective. Nanotechnology could help researchers target the limited number of surface receptors on tumor cells. Only some receptors make good targets, but they do not cover the entire surface of every tumor cell. Moreover, receptors continually turn over, with old ones being broken down and new ones being constructed, which further limits the availability of such receptors as sites for drug actions. The solution for overcoming these limitations could be to combine tumor-targeting peptides with drug-bearing nanoparticles. The best resolution for scanning electron microscopy (SEM) is usually about 500 nm, but using nanoparticles and a nanoscale view, like the one provided by field emission scanning electron microscopy (FESEM), enables a best resolution that is 100 to 1,000 times smaller. A new imaging modality called Magnetic Particle Imaging (MPI) is at an exciting stage of development like where MRI was in the early 1980s, when commercial MRI scanners and contrast agents were just being developed. In recent years, steady progress has been made with pre-clinical imaging for a variety of biomedical applications. MPI has the potential to be several hundred times more sensitive than MRI in detecting the nanoparticles. MPI has prospective clinical utility since it would provide opportunities for kidney-safe angiograms, stem cell tracking in vivo, contrast studies to diagnose and stage cancer, and inflammation imaging in vivo. In the future, the technique may be used in cardiovascular diagnostics.

Nanoparticles in Biological Imaging Srecko Gajovic

Applications of nanoparticles, quantum dots, in diagnostics, biomarkers, cell labeling, contrast agents for biological imaging, drug delivery systems, and anticancer nanodrugs for treatment of cancer and other infectious diseases.

Nanoparticles that can be detected by six medical imaging techniques:

- computed tomography (CT) scanning;
- positron emission tomography (PET) scanning;
- photoacoustic imaging;
- fluorescence imaging;
- upconversion imaging; and
- Cerenkov luminescence imaging.

https://phys.org/news/2015-01-nanoparticle-medical-imaging.html#jCp

Biological applications of nanocrystals Artur Podhorodecki – Rosttyslav Bilyy

Nanocrystals capable of sensing at the single-molecule level in living cells, and capable of parallel integration for detection of multiple signals, enabling a diversity of simultaneous experiments, as well as better imaging with the microscope and in *ex vivo* or *in vivo* studies. Quantum dots (QDs) bridge the gap between the solid state and single atoms, and hence these specks of matter exhibit a mix of solid-state and atomic properties.

Role of Magnetic Iron Nanoparticles in Life Sciences and Medical Imaging Daniel Horak

Magnetic nanoparticles are well-established nanomaterials that offer controlled size, ability to be manipulated externally, and enhancement of contrast in magnetic resonance imaging (MRI).

Because of the potential benefits of multimodal functionality in biomedical applications, researchers would like to design and fabricate multifunctional magnetic nanoparticles. As a result, these nanoparticles could have many applications in biology and medicine, including protein purification, drug delivery, and medical imaging. Currently there are three strategies to fabricate multifunctional magnetic nanoparticles.

1., Molecular functionalization, that involves attaching antibodies, proteins, and dyes to the magnetic nanoparticles. After their conjugation with proper ligands, antibodies, or proteins, the biofunctional magnetic nanoparticles exhibit highly selective binding.

2., Integration of the magnetic nanoparticles with other functional nanocomponents, such as quantum dots (QDs) or metallic nanoparticles. Because they can exhibit several features synergistically and deliver more than one function simultaneously, such multifunctional magnetic nanoparticles could have unique advantages in biomedical applications.

3., Paramagnetism, that exhibits features such as fluorescence or enhanced optical contrast. The hybrid nanostructures could provide a platform for enhanced medical imaging and controlled drug delivery.

Controlled drug delivery and reduced side effects of cancer drugs with nanocarriers Rostyslav Stoika, Roman Lesyk, Rostyslav Panchuk and Lesya Kobylinska

Balance between the anticancer activity of novel and/or traditional drugs and the oxidative stress is required to study the side effects. The expression of negative side effects strongly depends upon free radical oxidation or reactive oxidant species (ROS) balanced processes and the activity of the antioxidant system (AOA). Nanoparticles of various sizes and chemical composition can enter into cells and into mitochondria. This can lead to disruption of the mitochondrial electron transduction chain, which leads to additional O2 production. Also, particles perturb the mitochondrial permeability transition pore, which leads to the release of pro-apoptotic factors and programmed cell death. The nanoconjugates could change the apoptosis mechanisms and could enhance the cytotoxic effect of the drugs conjugated to the nanoparticles.

Environmental impact of nanoparticles Tatiana Borisova and Sandor G. Vari

Nanoparticles can have harmful environmental effects, but they can also be helpful for the environment. The planet produces particulate matter that is lifted into air through volcanic eruptions. Ash released during volcanic eruptions is rich in magnesium, and any chemical element existing as such or in one of its combinations may be found in the atmosphere in the form of nanoparticles during or immediately after eruptions. The Earth's large deserts are a major permanent source of nanoparticles which air currents lift into the atmosphere. The black carbon nanoparticles (rBC) emitted to the atmosphere through brush or forest fires have significant impact on human health. The main harmful effects of natural nanoparticles are the pro-inflammatory effects. They include interleukin-1 (IL-1), IL-12, and IL-18, tumor necrosis factor (TNF), interferon gamma (IFN-gamma), and granulocyte-macrophage colony stimulating factor and play an important role in mediating the innate immune response. Inflammatory cytokines are predominantly produced by and involved in the upregulation of inflammatory reactions. Major anti-inflammatory cytokines include IL-1 receptor antagonist, IL-4, IL-6, IL-10, IL-11, and IL-13. Specific cytokine receptors for IL-1, TNF-alpha, and IL-18 also function as proinflammatory cytokine inhibitors. The uncontrolled presence of natural carbon containing nanoparticles may pose a risk for the central nervous system in neurotransmission and could initiate neurodegeneration. A balance between proinflammatory and anti-inflammatory cytokines is necessary to maintain health.

ABSTRACTS

RECOOP-CSMC Senior Scientist Award Competition

The role of obesity-induced low-grade inflammation in adipokine signaling in the pregnant rat uterus

Applicant scientist: Róbert Gáspár gaspar@pharm.u-szeged.hu
Applicant organization: Department of Pharmacodynamics and Biopharmacy, University of Szeged, Szeged, Hungary

Key words: obesity, pregnancy, uterus, adipokines, low-grade inflammation

Aim of the project: Our aim is to investigate the mechanism of obesity-induced low-grade inflammation (OILGI) in the alteration of uterine and cervical responses to adipokines in the obese pregnant rat.
Background: In our previous obese pregnant rat study we proved that obesity reduced the fetal and placental weights and altered the contractility response: in non-obese pregnant uteri leptin and kisspeptin induced a moderated relaxation, while adiponectin elicited contractions. The uterine expressions of kisspeptin and leptin were increased; however the adiponectin receptor remained unchanged. These findings are in harmony with the earlier findings that leptin is a pro-inflammatory adipokine while placental kisspeptin has a proapoptotic effect during pregnancy on placental development. OILGI itself reduced the cervical resistance in pregnant rats. Kisspeptin and adiponectin reduced the cervical resistance in non-obese pregnant rats, but their actions were ceased in obesity. Contrarily, leptin was ineffective on non-obese cervical resistance but elicited resistance reducing action in obese cervices. These results indicate that the signaling of the adipokine receptors may be altered by OILGI.
Coherence with RECOOP Research Strategy: Our project plan tightly fit the goals of the Common Mechanism of Diseases and Reproductive Health research platforms of RECOOP.
Outline of the project: During the 24 months of the project we will focus on the link between OILGI and altered uterine cervical responses in the obese pregnant rat. We intend to measure the plasma, placental and visceral fatty tissue alterations of adipokines (leptin, adiponectin, kisspeptin), sexual hormones, inflammatory cytokines, and plasma ratio of pro-inflammatory/anti-inflammatory fatty acids. We will investigate the apoptotic capsase-3 activity in placental, uterine and fatty tissues. Special attention will be given to the signaling of adipokine receptors in uterine tissue. Finally, we will try to reduce the consequences of OILGI with procyanidin pretreatment, since procyanidins are proven~~ed~~ to inhibit the intensity of OILGI by alteration of inflammatory cytokines.
Expected outcome: We will gain elaborate insight of the obesity and LGI-induced apoptotic process in obese pregnant rats and gain further data about the cooperative network of hormonal, adipokine and fatty acid regulation involved in the pathomechanism. If we can reduce or prevent the obesity-induced alterations in uterine responses by procyanidins, we can provide functional evidence for the role of OILGI in pregnant uterine function and fetal development.

Ethical Committee or Institutional Animal Care and Use Committee Approval: All experiments involving animal subjects are approved by the Hungarian Ethical Committee for Animal Research (permission number: IV./3071/2016).
Acknowledgement: We thank Cedars-Sinai Medical Center's International Research and Innovation in Medicine Program, the Association for Regional Cooperation in the Fields of Health, Science and Technology (RECOOP HST Association) for their support of our organization as participating Cedars – Sinai Medical Center - RECOOP Research Centers (CRRC).

Deciphering the role of NET formation in NASH pathogenesis and testing of potential preventive drug candidates.

Applicant scientist: Rostyslav Bilyy
Applicant organization: Danylo Halytsky Lviv National Medical University

Non-alcoholic fatty liver disease, eventually resulting in non alcoholic steatohepatitis (NASH), are severe conditions affecting 25% and 5% of the population, respectively. The only available animal model is the high-fat high-cholesterol diet (HFHCD) model (Nature Medicine editorial, 2017). Earlier we established a unique mouse model of NASH using the C57BL/6NJ mice strain, bred at Lviv National Medical University and fed HFHCD.
While studying this animal model we observed a strong relation between NASH formation and neutrophil activation / formation of neutrophil extracellular traps (NET):

A. Neutrophils patrol ducts. The liver is near the bacteria-filled intestine, as well as the pancreas, for which extensive neutrophil patrol may result in intraductal NET aggregates that occlude the pancreatic duct (Leppkes, 2016).
B. Interaction of small hydrophobic nanoparticles with the cell plasma membrane results in its damage, leading to neutrophil activation and NET formation (Muñoz, Bilyy et al., 2016); this is also true for cholesterol nanocrystals (Desai, 2017), and sterile inflammatory conditions (Biermann, 2016).
C. We recently demonstrated (Nature Medicine, 2018, revision 1, submitted) that NET serves as a nucleation center for gallstone formation. Subsequent cholestasis is known to cause hepatocyte damage (Cai and Boyer, 2017) and liver inflammation (Allen, 2011) due to bile acid action (Li, 2017).

The current project is aimed to test a few approaches for preventing NET formation in their ability to influence liver damage induced by HFHCD. We plan to employ histological analysis of liver tissue, using conventional H&E staining, detection of lipids and collagen fibers, as well as immune histochemistry for externalized DNA and NET formation to investigate the following conditions:

1. Determine the dynamics of NASH in the mouse during HFHCD and the implication of neutrophils in each stage.

2. Evaluate NASH in the mouse during HFHCD and conditions interfering with NET formation with the aim to stop NET-related liver damage. Namely, by 2a) - specific neutrophil depletion with 1A8 antibodies (Daley, 2008); 2b) - block of polymorphonuclear cell PMN migration with metoprolol (García-Prieto, 2017); block of NET formation with 2c) - heparin (Fuchs et al., 2010); 2d) - ROS scavengers, like NAC, rutin trihydrate (D'Amico, 2013; Kirchner, 2013); and 2e) - PAD4 inhibitor Cl-amidine (Subramanian, 2015).

Obesity-induced cooperation of BMP and Wnt signaling pathways in the mediation of uterine myometrium apoptosis.

Applicant scientists: Olexandr Korchynskyi, Ph.D (PI) and Robert Gaspar, Ph.D. (co-PI).

Applicant organization: Institute of Cell Biology, Natl. Acad. Sci, Lviv, Ukraine

Background. The increasing prevalence of maternal obesity is a major public health concern for modern society. It is associated with increased incidence of caesarean section, pregnancy induced hypertension, and postpartum hemorrhage. Apart from its major interference with hormonal balance, obesity reduces fertility, and in pregnancy it is associated with a heightened risk of gestational diabetes mellitus and failure of uterine contractions. It was shown in rat models, that gestational obesity increases the intensity of spontaneous contractions of the last-day pregnant uterus but reduces uterine sensitivity to oxytocin. Disturbed hormonal balance can also lead to dysregulation of adipokine, Wnt/β-catenin and bone morphogenetic protein (BMP) activation signals resulting in ~~a~~ pregnancy pathology and diseases in the offspring. A deeper understanding of the molecular mechanisms underlying the adverse effect of maternal obesity on pregnancy is desperately needed for the generation of novel strategies to prevent and treat of obesity-provoked developmental abnormalities.

Preliminary Data: Our preliminary studies performed in collaboration with Dr. Robert Gaspar's lab at Szeged University, Szeged, Hungary showed that obesity induced by a high fat-high sucrose diet increases the level of BMP-2and tend to increase levels of Inhibitor of DNA-binding/differentiation protein (Id1) and 2 proteins and the phosphorylated form of Smad1/5/8 protein in the placenta of obese rats. In parallel, obesity caused fetal and placenta growth retardation in our experimental animals. Dysregulation of the Id1 protein may also affect the formation of new blood vessels. Id1 and Id3 are also required for neurogenesis, angiogenesis and vascularization of tissues (Lyden et al., 1999; Roschger and Cabrele, 2017). Thus, Wnt, BMP, Smad and Id proteins are important regulators during early development and are also implicated as direct causes for defects in bone formation, angiogenesis, tumor formation and neurodevelopmental disorders.

On the other hand, multipotent mesenchymal stem cells are capable of differentiating into myoblasts, osteoblasts, chondroblasts and adipocytes depending on the instructive signals to which they are exposed. Several studies have demonstrated a positive crosstalk between Wnt and BMP pathways in osteoblasts. Our preliminary data allow us to hypothesize a possible existence in the uterine myometrium of previously unrecognized obesity-induced cooperation between BMP and Wnt signaling pathways that can result in induction of Id protein expression on the way to inducing mitochondria-mediated apoptosis.

Study plan. Obesity in experimental female rats will be induced using a standard high fat-high sucrose diet. Blood and tissues samples (placenta, myometrium, newborn pups) will be collected from the obese and normal body weight females, and in the case of blood samples from newborns pups - using the method of decapitation. Correlative investigation of BMP-Smad1/5/8 phosphorylation, Id1/2/3 expression and the level of apoptosis detected with the TUNEL assay in rat tissues will be performed using immunohistochemical analysis. In parallel, we will perform *in vitro* investigation of the possible positive cooperation between BMP and Wnt signaling pathways in the uterine myometrium as a way to induce apoptosis in primary and established cell lines derived from human myometrium, with consequent studies of the molecular mechanism that mediates such crosstalk.

Expected outcomes: Our preliminary data suggest the possibility that induction of Id protein expression resulting from previously unrecognized obesity–induced positive cooperation between BMP and Wnt signaling pathways could serve as the key mediator in mitochondria-mediated apoptosis in the uterine myometrium.

Ethical Committee Approval: All experiments with animals and human subjects will be performed under approval from the Institutional Review Boards of the Institute of Cell Biology, Natl. Acad. Sci, Lviv, Ukraine and Szeged University, Szeged, Hungary in strict compliance with the rules for humane treatment of experimental animals and patient consent.

RECOOP
RESEARCH IN PROGRESS
Part I

Oral Presentations

Connexin 37 gene polymorphism and atherosclerotic changes in diabetic women type 1 and 2.

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Key words: connexin 37 gene, diabetes mellitus, women, atherosclerosis

Background: The gene of connexin 37 (C1019> T (Pro319> Ser)) is considered a candidate gene for cardiovascular disease. However, controversial data in epidemiological studies have been obtained regarding which particular polymorphism is of risk. Among strong modifiers could be traditional risk factors, mainly diabetes mellitus. Diabetes, however, presents in two main different forms (type 1 and 2) with potential different effects on the vascular wall. The aim of our study was to assess if the effect of connexin 37 gene polymorphism on the artery wall could be modified by the type of diabetes.
Methods: We analysed the association between the connexin 37 gene and preclinical atherosclerosis detected by high-resolution ultrasound in carotid arteries defined as a Belcaro score more than 2 in women with type 1 (n=305) and type 2 (n=128) diabetes.
Results: In women with diabetes type 2, all traditional risk factors (age, smoking, dyslipidemia, high blood pressure) were found to be significantly less favourable than in women with diabetes type 1 ($p<0.001$). Women with diabetes type 1, T allele carriers, had significantly higher prevalence of preclinical atherosclerosis than CC homozygotes (27.5 vs. 19.4 %; $p=0.026$). In contrast, women with diabetes type 2, T allele carriers had significantly lower prevalence of preclinical atherosclerosis than CC homozygotes (47.1 vs. 63.3 %; $p=0.029$). No differences in other main cardiovascular risk factors including smoking, hypertension, renal disease and dyslipidemia between T allele carries and CC homozygotes in both groups were detected.
Discussion and Conclusion: We detected significant positive association between the T allele of the gene for connexin 37 and atherosclerosis in women with diabetes type 1, but the opposite finding was detected in women with diabetes type 2. This difference could be explained by interaction of the gene for connexin 37 with different spectrum of risk factors in both types of diabetes including different duration of hyperglycemia.
Source of research: Supported by MH CZ - DRO ("Institute for Clinical and Experimental Medicine – IKEM, IN 00023001") and by MH CZ - conceptual development of research organization (Motol University Hospital, Prague, Czech Republic 00064203).
Ethical Committee Approval: This project was supported through the Internal Grant Agency of the Ministry of Health, Czech Republic NT 14008-3/2013 and under this number it was approved by local Ethical Committee in 2013. Then it was repeatedly approved by the local Ethical Committee in 2016/2017.
Acknowledgement: We thank Cedars - Sinai Medical Center's International Research and Innovation in Medicine Program, the Association for Regional Cooperation in the Fields of Health, Science and Technology (RECOOP HST Association) for their support of our organization as participating Cedars – Sinai Medical Center - RECOOP Research Centers (CRRC).

Influence of Different Diets on Fatty Acid Composition in Plasma of Rats

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Keywords: Free fatty acids, obesity, plasma,

Introduction: Plasma free fatty acids (FFA) play important physiological roles in many organs. However, chronically elevated plasma FFA levels appear to have pathophysiological consequences. Recent data indicate that high plasma FFA levels have a significant role in contributing to insulin resistance.
Our aim was to investigate changes of fatty acid composition (inflammatory and anti-inflammatory) in the plasma of male and female rats, after feeding with different diets.
Methods: Plasma samples were obtained from male and female rats fed: 1. Standard diet (normal) 2. High fat-high sucrose diet (HFHS). 3. HFHS + Metformin. 4. HFHS + Liraglutide
***Sample preparation*:** A modified method was developed based on Kopf and Schmitz (J. Chromatogr. B 938, 22-26, 2013). Lipids and FFA were extracted with n-hexane from plasma samples using a Vortex mixer. Extracted lipids and FFA were then separated using the solid phase extraction (SPE) method. All separated components were converted to volatile methyl esters (FAMEs) using a boron-trifluoride methylation procedure.
***Gas Chromatography*:** Fatty acid methyl esters were determined by gas chromatography (GC) using flame ionization detection (FID).
Results: To investigate the development of insulin resistance with fatty acids we focused on determining the following fatty acids: among the anti-inflammatory fatty acids oleic acid (C18:1, n-9) and linoleic acid (C18:2, n-9) were the main FFA detected; lauric acid (C12:0) and caprylic acid (C8:0), erucic acid (C22:1, n-9), capric acid (C10:0), alpha-linolenic acid (C18:2, n-3), and gamma-linoleic acid (C18:3, n-6) were found in small amounts. Palmitic acid (C16:0) occurred in high concentration among the inflammatory fatty acids. Among the above fatty acids, stearic acid was also detected in high concentration.
Discussion and Conclusion: The developed SPE–GC method allows a rapid separation of the FFA-fraction from a neutral lipid extract of plasma taken from rats. The different diets caused significantly different fatty acid compositions in plasma according to sex, feeding and drug treatment.

Funding: The study was funded by a RECOOP HST grant.
Acknowledgement: This study was supported by Cedars-Sinai Medical Center's International Research and Innovation in Medicine Program, the Association for Regional Cooperation in the Fields of Health, Science and Technology (RECOOP HST Association) and the participating Cedars – Sinai Medical Center - RECOOP Research Centers (CRRC).
Ethical Committee Approval: Hungarian Ethical Committee for Animal Research: registration number IV/3796/2015.

Correlation between placental volume measured by ultrasound *in-vivo*, and sFlt-1/PlGF ratio in preeclampsia

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Keywords: placental volume, sFlt-1/PlGF ratio, preeclampsia, VOCAL

Introduction: The aim of the present study was to identify the correlation between placental volume measured by 3-dimesional (3-D) ultrasound *in-vivo*, and the ratio of soluble fms-like tyrosine kinase-1 (sFlt-1) to placental growth factor (PlGF) in preeclampsia (PE). We hypothesized a correlation between placental volume and the sFlt-1/PlGF ratio in PE. In angiogenesis sFlt-1 and PlGF are molecules of great importance and had correlation with placental vasculature and thus placental volume.
Methods: In our clinical research we recruited 73 pregnant women with increased risk for PE between January 2017 and September 2017 at the Department of Obstetrics and Gynecology in Szeged (HU) and Osijek (HR). Our prospective examinations detected the sFlt-1/PlGF ratio from maternal venous blood samples (every four weeks between 20th and 36th), in parallel with *in-vivo* placental volume measurements in pregnancies at risk for PE.
Results: Average *in-vivo* placental volume (APV) measured at 20, 24, 28 and 32 weeks of gestation, and the sFlt-1/PlGF ratio in pregnancies with Previous PE (PrevPE), chronic hypertension (CHT), gestational hypertension (GHT), pregestational diabetes mellitus (PreDM) and PE are presented in *Table 1*.

Table 1.	**Weeks of gestation**	**PrevPE** (n=18)	**CHT** (n=28)	**GHT** (n=7)	**PreDM** (n=9)	**PE** (n=11)
APV *in-vivo* (mean cm^3)	20	227.90	231.45	192.33	243.37	117.48
	24	248.71	254.36	228.66	287.33	136.55
	28	258.11	283.40	245.76	393.50	n.d.
	32	336.20	468.51	337.51	560.55	199.53
sFlt-1/PlGF ratio (mean±SD)		9.1±5.8	5.7±3.4	9.4±9.8	5.8±3.3	349±140*

Discussion: In our results, placental volume was significantly ($p<0.001$) lower in PE compared to the other groups examined, as expected. We found that the higher the sFlt-1/PlGF ratio is, the lower the placental volume will be in the case of PE.
Conclusion: Our results support the hypothesis, that there is correlation between placental volume and increased sFlt-1/PlGF ratio in PE. These results may help to understand the underlying pathomechanisms in PE and to successfully predict potential adverse pregnancy outcomes.
Source of research support: The study was supported by BM CSMC - RECOOP Young Scientists Research Grant 2016.
Ethical Committee Approval: University of Szeged (No.: SZTE 32/2014).
Acknowledgement: We thank Cedars-Sinai Medical Center's International Research and Innovation in Medicine Program, the Association for Regional Cooperation in the Fields of Health, Science and Technology (RECOOP HST Association) for their support of our organization as a participating Cedars-Sinai Medical Center - RECOOP Research Center (CRRC).

Medicinal Chemistry of 4-Thiazolidinones: New Challenges and Solutions

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Key words: drug design, 4-thiazolidinones, synthesis, biological activity, Michael acceptor

Introduction: 4-Thiazolidinones as privileged heterocycles possess a variety of biological activities (in both screening campaigns and directed experiments). They are confirmed selective ligands toward various biotargets as well as compounds with unknown mechanisms of actions. Currently many thiazolidinones, especially 5-ene-derivatives, have been claimed as PAINS or frequent hitters, mainly due to Michael acceptor functionality. This is discussed and very often applied in a non-correct manner; additionally following the new trend, such Michael acceptor functionality can be useful in the creation of new drug-like molecules.
Methods. Synthesis, biological assays, SAR analysis.
Results and discussion. Following the previous data the in-house library of new heterocycles has been improved. The new synthetic protocols for transformations of the thiazolidinone frame were developed based on combinatorial approach, bioisosteric replacement, molecular hybridization, privileged substructure-based diversity-oriented synthesis etc. Screening of biological activity of synthesized compounds led to focuses of these main areas: search of anticancer, antiparasitic/antimicrobial agents and compounds able to decrease oxidative stress as a key pathogenetic mechanism in many disorders, including the above-mentioned. Moreover, following the polypharmacological approach and the concept of multi-target drug design, the compound possessed several pharmacological effects e.g. simultaneous anticancer and antitrypanosomal activities regarded as an advantage in the development of new hits. Following the activity of the products of thiazolidinone transformations, they can be considered as pro-drugs with efficient characteristics. SAR analysis allowed the outlining of the direction for 4-thiazolidinones optimization: complication of C5 fragment and modification of N3; the isosteric replacement; combination of thiazolidinone core with other pharmacologically attractive fragments; annealing in complex heterocyclic systems; thiazolidinone-based synthesis of other heterocycles. Additionally Michael functionality of some thiazolidinones should be utilized in the useful way (Michael acceptors are activators of Nrf2; covalent inhibitors of set of biotargets; modulators of ROS-dependent pathways). For active anticancer thiazolidinones a PPAR-mediated, ROS-dependent and proapoptotic mechanism of action was proposed that required further in depth study and search for possible targets. The thiazolidinones as structural mimics of H_2S donors should be studied as potent H_2S releasing agents that open new opportunities for their usage.
Conclusions. 4-Thiazolidinones remain simple and effective tools for creation of new drug-like molecules. The new properties of thiazolidinone-based compounds should be analyzed in depth and used in the useful way but not be regarded as useless *per se.*
Acknowledgement: We thank Cedars-Sinai Medical Center's International Research and Innovation in Medicine Program, the Association for Regional Cooperation in the Fields of Health, Science and Technology (RECOOP HST Association) for their support of our organization as participating Cedars-Sinai Medical Center-RECOOP Research Centers (CRRS).

Small but smart actors in big performance - the nanomedicines

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Keywords: nanomaterials, anticancer drugs, mechanisms

Introduction. The last two decades demonstrated a real burst in investigations dedicated to the creation of novel nanomaterials for biology and medicine, as well as in the development of the overlapping fields such as agriculture, ecology, and the cosmetic industry. If the early nanomaterials for biomedical application possessed limited functional properties, nowadays, scientists create multifunctional nanomaterials that are, for example, capable of improved drug delivery, the bio-imaging needed for monitoring the addressed delivery and clearance, circumventing biological barriers in the organism, providing sensitivity to specific stimuli, and others. Thus, new terms have appeared such as "**intellectual**" or "**smart**" drugs, "**molecular nano-robots**", etc. It is desirable that "smart" drugs and platforms used for their delivery possess organic structure that is biocompatible and biodegradable. While various diagnostic remedies for monitoring the pathological state may possess a mineral basis, there are exceptions to that rule.

Results and Discussion. Here we described a strategy of the creation, physico-chemical characteristics, and biomedical application of several **"true nanomaterials"** whose nanosize (<100 nm) scales provides them with new important characteristics. Such "truthfulness" of the developed drug-functionalized nanoplatforms was responsible for: **a)** enhanced (up to 10 times and more) effectiveness of their antineoplastic (pro-apoptotic) action *in vitro* and therapeutic (anticancer) action *in vivo*; **b)** significant acceleration of drug delivery to target cells; **c)** circumventing natural biological barriers, for example, the multi-drug resistance in tumor cells; **d)** significant reduction of adverse side effects of the anticancer drugs in the treated organism, such as cardio-, hepato- and nephro-toxicities; **e)** prolongation of stability of anticancer drugs and of their treatment effect; **f)** providing water solubility to water insoluble drug substances that makes easier their application in chemotherapy. These properties have been verified ~~at~~ using both traditional (doxorubicin, cisplatin or stage 2 clinical trial metal-containing KP-1019 drug) and experimental (4-thiazolidinone and thiazol derivatives, antibiotics of landomycin family) anticancer drugs, as well as various nanoplatforms for their delivery (VEP-GMA -graft-PEG polymer functionalized with phospholipid, Fullerene C60, and other polymeric nanoplatforms whose investigation has been started recently).

Conclusions. 1) The number and impact factor of published papers devoted to development and biomedical applications of novel multifunctional nanomaterials at the ICB are stably increasing. 2) Collaborations of the ICB with organizations of the RECOOP-HST Association (Institute of the Macromolecular Chemistry in Prague, Czech Republic), Danylo Halytsky Lviv National Medical University in Ukraine), as well as the non-RECOOP-HST organizations (Lviv National Polytechnic University, Taras Shevchenko Kyiv National University, both in Ukraine) are productive and developing.

Acknowledgements. Cedars-Sinai Medical Center's (CSMC) International Research and Innovation in Medicine Program; Association for Regional Cooperation in the Fields of Health, Science and Technology (RECOOP-HST Association); Participating Cedars–Sinai Medical Center - RECOOP Research Centers (CRRC).

Imaging parameters of repair after ischemic brain lesion in the mouse

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Keywords: stroke, TLR2, Gap43, neuroinflammation, in vivo imaging

Introduction: The mouse model of the human ischemic stroke includes temporary occlusion of the medial cerebral artery (MCAO) followed by reperfusion. After the acute phase of the detrimental consequences of ischemia, brain damage occurs, which is subsequently counteracted with repair processes in the chronic phase. The possible therapeutic approaches can target diverse consequences of ischemia, being oriented to neuroprotection or repair stimulation. Subsequently, the in vivo imaging can serve to monitor and evaluate the consequences of the brain ischemia.
Materials and methods: MCAO for 60 minutes followed by reperfusion was performed in 3-month old mice. The ischemic lesion was evaluated by magnetic resonance imaging (MRI, Bruker 7T Biospec 70/20 USR) and bioluminescence imaging (BLI, Perkin Elmer IVIS Spectrum). Tlr2, Casp3 and Gap43 were used as molecular markers. Tlr2 loss of function mice were used as a model for modified neuroinflammation after ischemic lesion. The imaging data were complemented by functional tests and Western blot protein analysis of the brain samples.
Results: Gap43 was used to evaluate elements of repair after MCAO. Its expression correlated with the ischemic lesion measured by MRI. Modifying the neuroinflammation in Tlr2 loss of function mice had as a consequence increased Gap43 and Casp3 activities, accompanied by an increase in synaptogenic markers.
Discussion and Conclusion: Bioluminescence imaging combined with magnetic resonance imaging can serve as a multimodal approach to assess the elements of brain repair after ischemic lesion in the mouse.

Ethical approval: University of Zagreb, School of Medicine no.: 380-59- 10106-17- 100/187.

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Conjugation of 4-thiazolidinone derivatives with PEGylated nanocarrier enhances their treatment effect, improves water solubility, and reduces general toxicity in tumor-bearing mice

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Keywords: 4-thiazolidinone derivatives, mice BALB/C, lymphoma NK/Ly, apoptosis

Introduction. To study: 1) treatment effect of 4-thiazolidinone derivatives (Les-3288, Les-3833) in NK/Ly lymphoma grafted to BALB/C mice; 2) enzymatic and cytological indicators of general toxicity during the effect of these compounds in lymphoma-bearing mice; 3) providing water solubility to studied 4-thiazolidinone derivatives by their conjugation with PEG-containing polymeric nanocarrier (PNC); 4) enhancing of pro-apoptotic action of these compounds in rat glioma C6 cells *in vitro*.

Methods. *In vitro* experiments were performed with rat glioma C6 cells and evaluated changes in their nativity, as well as cell cycling pattern and annexin V expression. PNC was and applied for providing water solubility to 4-thiazolidinone derivatives and enhancing their pro-apoptotic action towards glioma C6 cells. BALB/C mice implanted with NK/Ly lymphoma were treated for 14 days with doxorubicin (Dox, 1 mg/kg), Les-3833 (2.5 mg/kg) and Les-3288 (5 mg/kg). Therapeutic effects were tested in 30 days of tumor growth. Lymphoma development was monitored by measuring the amount of ascites in the treated mice.

Results. Apoptosis was shown to be the mechanism of killing rat glioma C6 cells *in vitro*. Such mechanism was confirmed by FACS analysis of the number of cells in pre-G1 stage, the amount of annexin V positive and propidium iodide negative C6 cells, as well as the ratio of cells with condensed and fragmented choromatin. Les-3833 and Les-3288 demonstrated the most pronounced treatment effect towards ~~to~~ the production ascitic fluid in BALB/C mice grafted with NK/Ly lymphoma. A distinct decrease in the amount of ascitic fluid was detected in the treated mice compared with the 1.5 times increase in its amount in the untreated (control group) mice. The activities of aspartate and alanine aminotransferases in blood serum were elevated at the 14^{th} day of animal treatment and returned to normal in 21 days. Dox induced severe anemia with a reduction in the number of red blood cells, while the 4-thiazolidinone derivatives were less toxic, and erythrocyte counts stayed normal after 21 days of treatment. The development of NK/Ly lymphoma led to an increase in neutrophil number, while the applied anticancer compounds reduced it significantly. Dox caused an increase in the number of lymphocytes in blood, while the 4-thiazolidinone derivatives did not change their count from the normal level.

Discussion and Conclusions. All studied substances killed tumor cells through apoptosis mechanisms. The treatment effects of novel 4-thiazolidinone derivatives towards NK/Ly lymphoma grafted to BALB/C mice were comparable to such effects of Dox. At the same time, the action of these derivatives led to fewer negative side effects measured as changes in number of erythrocytes, neutrophils and lymphocytes in blood and the activity of aspartate and alanine aminotransferases in blood serum, compared with the action of Dox. Conjugation of 4-thiazolidinone derivatives with novel PNC provided them with water solubility and enhanced their pro-apoptotic effects in rat glioma C6 cells.

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RESEARCH IN PROGRESS

Interactive Poster Presentations
Part I

Enhancing the effectiveness of low-level laser radiation with nanoparticles on clinical strains of *Staphylococcus aureus*

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Keywords: *Staphylococcus aureus,* low-level laser radiation, nanoparticles, oral cavity

Introduction: The growing resistance to antibacterial agents has brought on the need for other antimicrobial treatments. Low-level laser radiation (LLLR) has been proposed as an alternative treatment for localized bacterial infections in response to the problem of antibiotic resistance. The bactericidal activity of LLLR upon addition of Ag to TiO_2 is dramatically enhanced.
Methods: *S. aureus* cultures were added to the concentrations of nanoparticles (0,1, 0,5 and 1,0 mg/ml), follow by irradiation with a laser of blue spectra for 5 minutes.
Results: This study shows that TiO_2, Ag/TiO_2 and S/TiO_2 in combination with blue light had a phototoxic effect on *S. aureus*. Additionally, we observed that the LLLR effect was dose dependent for different nanoparticle concentrations. For species of *S. aureus*, elimination with efficacy was obtained with Ag/TiO_2 after irradiation with blue light.
Discussion: The current study revealed that Ag/TiO2, TiO2 and S/TiO2 particles irradiated with blue light, elicited lower cell viability as compared to non-irradiated particles. Nanoparticles with different concentrations also tended to show dose dependent effects. In the absence of blue light irradiaton was mainly responsible for the phototoxicity of Ag/TiO2, TiO2 and S/TiO2 particles.
Conclusion. The Ag/TiO_2 under blue light had better results for bactericidal ability in comparison with two other tested nanoparticles due to the photocatalytic property of TiO_2 and S/TiO_2 which did not have influence on killing of *S.aureus*. Analysis showed that Ag/TiO_2 particles were able to provoke more phototoxic effects in the presence of low-level laser radiation than TiO_2 and S/TiO_2.

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Ethical Committee or Institutional Animal Care and Use Committee Approval: 28/04/2014 № 4

Search for New Anticancer Agents Among Thiazole Derivatives with Michael Acceptor Functionality

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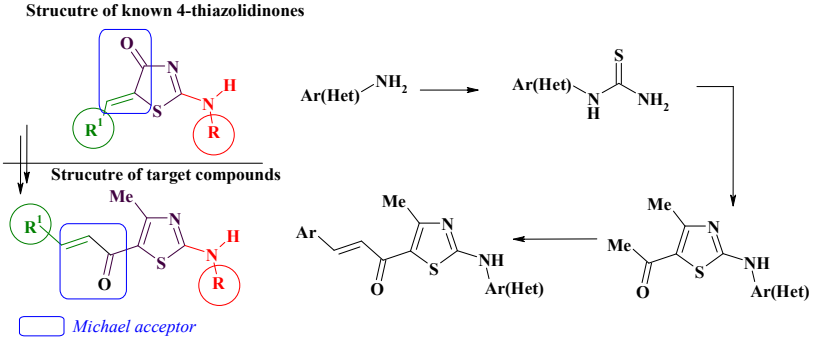
Keywords: 4-Thiazolidinones, thiazoles, synthesis, anticancer screening.

Introduction. Thiazolidinones are a well known class of heterocycles and efficient building blocks in the design of new drug-like molecules with various types of activity. Currently many thiazolidinones, mainly 5-ene-rhodanines, are assigned as PAINS (based on their possible Michael acceptor functionality) and are often treated as useless promiscuous binders. However, the useful properties of Michael acceptors in drug discovery are now again of special interest and Michael acceptors are considered as the "new old tool" for new drug creation, especially anticancer agents. Aiming to design new potent anticancer agents the structure of active 4-thiazolidinones were modified and the following points were taken into account: isosteric replacement of 4-thiazolidinone core by thiazole; the same localization and nature of the substituents in the basic core; presence of conjugated carbonyl group (Michael acceptor).

Methods. Structure-based drug design, wet-chemistry, anticancer screening.

Results and discussion. The synthetic protocol was developed based on the above-mentioned criteria within privileged substructure diversity oriented synthesis and involved [2+3]-cyclocondensation and Claisen-Schmidt condensation. The structure and purity of target 1-(4-methyl-2-R-aminothiazol-5-yl)-3-R-propenones were confirmed by the methods of ^{1}H, ^{13}C NMR-spectroscopy and mass-spectrometry. Screening of anticancer activity was performed against a panel of cancer cell lines representing different types of cancer in NCI, NIH within DTP program. The dose-dependent parameters were calculated.



A series of compounds with micromolar IC_{50} levels against cancer cell lines were identified. The hit-compounds showed low levels of toxicity. Following the obtained data the directions of further design were outlined. Additionally, target compounds possess antiexudative/anti-inflammatory activity that can be considered as one of the arguments for useful properties of Michael acceptor in drug design within the poly-pharmacological approach.

Conclusions. A row of thiazoles with Michael acceptor fragment in the molecules was designed and synthesized, the anticancer potency of target compounds was confirmed and the directions for further optimization were proposed.

Acknowledgement: We thank Cedars-Sinai Medical Center's International Research and Innovation in Medicine Program, the Association for Regional Cooperation in the Fields of Health, Science and Technology (RECOOP HST Association) for their support of our organization as participating Cedars – Sinai Medical Center - RECOOP Research Centers (CRRC). We are grateful to Dr. V.L Narayanan from Drug Synthesis and Chemistry Branch, National Cancer Institute, Bethesda, MD, USA, for *in vitro* evaluation of anticancer activity. The project was partly supported by State Fund for Fundamental Research of Ukraine (Ф76/72).

Ethical Committee Approval: Danylo Halytsky Lviv National Medical University Animal Care and Use Committee Approval: 18.03.2013, #3

Enhancement of water solubility and innate MDR-circumventing activity of landomycin A by its immobilization on poly(2-oxazoline) nanocarrier

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Introduction. Landomycin A (LA) is a novel anticancer antibiotic possessing a unique ability to circumvent cancer drug resistance, caused by different factors. However, its clinical applications are limited due to low solubility in water. Thus, the aim of this study was to prepare water-soluble pharmaceutical forms of LA by means of its immobilization on novel poly(2-oxazoline) nanocarriers and to study its impact on the cytotoxic potential of LA *in vitro*.

Methods. Evaluation of cytotoxic activity of LA nanoformulations was performed *in vitro* on a panel of 5 tumor cell lines overexpressing P-gp or MRP-1. ROS production was measured by flow cytometry using DCFDA (H_2O_2-specific) and DHE (O_2^--specific) dyes, induction of apoptosis was studied by annexin V/PI double staining, and cell cycle measurement by PI staining on BD FACScan flow cytometer.

Results. Immobilization of LA on poly(2-oxazoline) nanocarrier allowed the development of water-soluble solutions of this drug (2.0 g/L), which fully preserved its activity after continuous freeze/thaw cycles and solutions were stable for >30 days at RT. LA-polymer conjugates were found to produce 2-fold less H_2O_2 compared to LA in free form. However, such an antioxidant activity of poly(2-oxazoline) nanocarriers had no negative impact on the anticancer potential of LA. There was no difference in cytotoxic activity of LA nanoformulations compared to pure LA towards parental HL-60 leukemia and KB-3-1 carcinoma cell lines, while their drug-resistant sublines KBC-1 (P-gp+), HL-60/adr (MRP-1+), HL-60/vinc (P-gp+) were found to be 1,5 to 2-fold more sensitive to their action.

Discussion. High loading of LA on poly(2-oxazoline) nanocarrier and its long-term stability in water-based solutions allowed the override of the main drawback of this drug. The observed ability of nanocarrier to further enhance innate MDR-circumventing activity of landomycin A may be of crucial importance for effective treatment of disseminated drug-resistant tumors in cancer patients, while antioxidant properties of nanocarrier should decrease potential side effects of LA towards healthy tissues and organs. Further *in vivo* studies of LA-poly(2-oxazoline) conjugate on animal tumor models are in progress.

Conclusions. Immobilization of LA on poly(2-oxazoline) nanocarrier allowed the development of stable pharmaceutical forms of this drug, enhanced its MDR-targeting activity and decreased the production of potentially toxic H_2O_2 by LA. This may be of key importance for improvement of the anticancer potential of LA and lowering its side effects *in vivo*.

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4-Thiazolidinone derivatives change the membrane potential of rat brain nerve terminals

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Keywords: thiazolidinone, nerve terminals, membrane potential, new neuroactive compounds

Introduction: The design and synthesis of novel 4-thiazolidinones derivatives as potentially new neuroactive compounds and anticonvulsants was carried out. Leading compounds Les-2659, Les-2769, Les-3836, Les-3105, Les-2615, Les-2658, and Les-2670 were selected after *in vivo* screening for anticonvulsant activity. Here, we examined the effects of these leading compounds on the membrane potential of isolated rat brain nerve terminals (synaptosomes).

Methods: The effects of 4-thiazolidinone derivatives on the membrane potential were monitored using the potentiometric fluorescent dye rhodamine 6G (0.5 μM).

Results: No significant changes were found in the emission spectrum of rhodamine 6G after adding each of the investigated compounds at a concentration of 100 μM. Les-2659, Les-2769, Les-3105, and Les-2670 caused a weak depolarization of the plasma membrane of nerve terminals, increasing the fluorescence dye intensity by 5.42 ± 0.22 %, 5.74 ± 0.22 %, 5.11 ± 0.35 %, 3.36 ± 0.2 %, respectively. Les-3836 increased the fluorescence dye intensity by 19.35 ± 0.57 % ($p < 0.01$) and so, exhibited a more considerable depolarization effect on the membrane potential. Les-2615 and Les-2658 decreased the fluorescence intensity of rhodamine 6G by 10.24 ± 1.15 % ($p < 0.01$) and 15.98 ± 1.43 % ($p < 0.01$), respectively, reflecting hyperpolarization of the plasma membrane of the synaptosomes.

Discussion and Conclusion: Therefore, the effects of novel 4-thiazolidinone derivatives on the membrane potential of nerve terminals were revealed. The plasma membrane potential is a crucial parameter, the changes of which can modulate transporter-mediated uptake/release and exocytotic release of neurotransmitters. Further comprehensive *in vitro* and *in vivo* studies of 4-thiazolidinone derivatives are necessary to prove their features as potential neuromodulatory drugs that can open new ways to combat neurological disorders.

Source(s) of research support This work was supported by DFFD # F76/72

Ethical Approval: Experiments were carried out in accordance with the European Guidelines and International Laws and Policies (Directive 86/609/EEC); the protocols were approved by the Animal Care and Use Committee of the Palladin Institute of Biochemistry (Protocol from 19/09-2012).

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

pH dependent fluorescence of CdSe/CdS nanoparticles in an aqueous phase

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Key words: hydrophilic nanocrystals, titration, quantum dots

Introduction: Surface ligands determine the interaction between colloidal nanocrystals (NCs) and their environment. One of the most important factors determining the quality of NCs is time stability of such ligand-NCs complexes, which, moreover, depend on the pH of the solution. Thus, it is essential to select the proper pH value for the environment to obtain stable NCs-ligand complexes. It is generally known that the pH of the medium influences the adsorption/desorption of the ligand layer and thus, modifies optical properties of NCs, such as photoluminescence (PL) intensity. The pH value is the key factor for all biological fluids. Consequently, studies of the optical properties of NCs in a different pH are the important issue from the application point of view, e.g. when developing assays, bioprobes and biosensors based on NCs. Progress, in this respect, requires a better understanding of the surface-ligand and ligand-ligand interactions at the NCs-ligand interface.
Methods: We used the ligand-exchange method to transfer the hydrophobic NCs to aqueous media. The pH value of the NCs colloidal solution was controlled by titration of acid/base. The changes in NCs optical properties were monitored with PL measurements.
Results: We obtained the hydrophilic complexes of NCs-ligands having the highest PL intensity in pH~4 conditions. PL in pH=4 is 1.5 times higher as compared to the standard physiological conditions (pH~8) and over 2 times higher as compared to alkaline conditions (pH ~ 9-12). Nevertheless, the NCs-ligands complex was found to be stable for at least 30 days in all examined pH conditions.
Discussion: The changes in pH of the colloidal solution influence the charge at the NCs surface. This phenomenon is related to the ligand functional groups. The carboxylic group of the ligand is responsible for PL intensity changes in the acidic conditions, while the thiol group in the alkaline environment.
Conclusion: The PL intensity of hydrophilic NCs depends on the charge state of the surface ligands, which is related to the pH condition of the colloidal solution. For hydrophilic NCs (prepared by our procedure) we found that in a weakly acidic (pH ~ 4) environment the NCs have the highest PL intensity, while in the alkaline conditions PL intensity decreases about 30%.

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Polymer-coated upconversion and magnetic nanoparticles for biomedical applications

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Keywords: upconversion; magnetic; radiolabeling; bioimaging; photodynamic therapy.

Introduction: Design of versatile "all-in-one" nanoprobes with excellent optical and magnetic properties that can simultaneously integrate both diagnostic and therapeutic functions is one of the main challenges for current biomedical research.

Methods: Upconversion and/or magnetic $NaYF_4:Yb^{3+}/Er^{3+}$ or $NaGdF_4:Yb^{3+}/Er^{3+}$ nanoparticles were prepared by high-temperature co-precipitation of lanthanide chlorides. To enhance biocompatibility and colloidal stability in water and physiologically relevant buffers, the particle surface was modified by inorganic and organic polymers resulting in $NaY(Gd)F_4:Yb^{3+}/Er^{3+}@SiO_2$ and $NaY(Gd)F_4:Yb^{3+}/Er^{3+}$@PEG nanoparticles, respectively. The biocompatibility of the $NaGdF_4:Yb^{3+}/Er^{3+}@SiO_2$ particles was tested *in vitro* using mouse 3T3 fibroblasts and B16F10 melanoma cells. Nanoparticles were intravenously administered in B16F10 melanoma bearing mice and particle localization was evaluated *ex vivo* in tumor, liver, and brain tissues using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). The surface of the $NaY(Gd)F_4:Yb^{3+}/Er^{3+}$@PEG nanoparticles was also labeled with radioiodine for facile *in vivo* biodistribution studies in non-tumor mice by single-photon emission computed tomography (SPECT) and/or magnetic resonance imaging (MRI). For noninvasive NIR-induced photodynamic therapy (PDT), Al carboxyphthalocyanine (Pc) was conjugated to the $NaYF_4:Yb^{3+}/Er^{3+}@SiO_2$ nanoparticles, which were intratumorally administered into mammary carcinoma MDA-MB-231 bearing athymic nude mice.

Results: The upconversion/magnetic nanoparticles proved to be non-toxic at moderate concentrations. The $NaGdF_4:Yb^{3+}/Er^{3+}@SiO_2$ and ^{125}I-labeled $NaY(Gd)F_4:Yb^{3+}/Er^{3+}$@PEG particles were localized in blood vessels and liver, respectively. Upon NIR irradiation (980 nm), the red emission of the Pc-conjugated $NaYF_4:Yb^{3+}/Er^{3+}@SiO_2$ nanoparticles quenched, confirming effective energy transfer from the particles to Pc, which generates cytotoxic singlet oxygen ($^1O_2$). Extensive tumor necrosis developed in all mice 24–48 h after PDT.

Discussion and Conclusion: Highly colloidally stable and biocompatible upconversion/magnetic nanoparticles were developed, demonstrating great promise as a novel bi-modal imaging tool and NIR-triggered PDT material for SPECT and MRI cancer theranostics.

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Semiconductor lead sulfide nanocrystals for infrared bio-medical imaging

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Key words: semiconductor nanocrystals, quantum dots, bio-imaging, infrared, lead sulfide

Introduction: Lead sulfide (PbS) semiconductor nanocrystals (NCs) exhibit tunable photoluminescence in the near infrared region (NIR) with high emission quantum yield and reduction of photobleaching. For these reasons they are attractive alternatives to conventional organic dyes, which are inefficient in the NIR range. Another advantage of NCs is the possibility of their surface engineering and coupling to various biological molecules such as proteins, antibodies or nucleic acids. In our recent studies we focused on optimization of the PbS NCs synthesis process, as well as on tuning NCs to have properties required for bio-medicinal imaging.

Methods: We used a standard wet-chemistry approach, in which the precursors of the semiconducting material (in this case lead and sulfur compounds) undergo thermal decomposition and crystalize in controlled conditions. Ligands such as oleic acid or other amphiphilic compounds are used to prevent aggregation and passivate the NCs surface. We proposed specific temperature conditions and novel composition of the reaction mixture that allowed us to improve the properties of the final products.

Results: Particles obtained with our method have diameters of several nm (usually between 3 and 7 nm), narrow size distribution and manifest good optical properties. The spectral position of the photoluminescence peak spreads through a broad wavelength range of the infrared region (from about 900 to 1600 nm). The shift of the emission wavelength is possible due to the quantum confinement effect, which occurs for sufficiently small NCs.

Discussion: NCs synthesized in our group have all the crucial characteristics needed for bio-medical imaging, thus we decided to test their performance in tissue imaging. For this purpose we built a customized microscopy system that is suitable for recording images and emission spectra in broad range (from approximately 400 to 1600 nm). We recorded fluorescence images of PbS NCs stained macrophages. The recorded images in infrared confirm the possibilities and show advantages of microscopy using our PbS NCs. We also investigated the blinking effect in the obtained PbS NCs, which involves switching photoluminescence between the on and off states. That kind of behavior in combination with restricted photobleaching is promising in the context of specific imaging techniques, e.g. localization microscopy.

Conclusion: We managed to develop a facile and repeatable method for synthesizing fine quality PbS NCs that can be tuned to have photoluminescence in the optical window for biological tissues and we demonstrated that our NCs are suitable for bio-imaging.

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Antioxidant core-shell iron oxide nanoparticles and their cellular interactions

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Keywords: magnetic nanoparticles; oxidative stress; carbohydrates

Introduction: Natural carbohydrates, as well as their antioxidant derivatives, such as glucose (Gl) and ascorbic acid (AsA), respectively, can enhance biocompatibility, cellular uptake, and colloidal stability of superparamagnetic iron oxide nanoparticles prepared by aqueous co-precipitation. The latter serve as contrast agents for magnetic resonance imaging or cell labeling, as well as carriers in controlled drug delivery systems or hyperthermia nanoheaters for tumor treatment.

Methods: Starting magnetic nanoparticles were prepared by aqueous precipitation and oxidation, resulting in formation of maghemite (γ-Fe_2O_3). To protect the iron oxide core from unwanted interactions, particles were coated with SiO_2 by tetraethoxysilane hydrolysis and surface-modified with glucose and ascorbic acid. Obtained nanoparticles were analyzed by means of transmission electron microscopy, infrared spectroscopy and dynamic light scattering. Antioxidant activity assay against hydroxyl and peroxyl radicals was performed by fluorescence intensity measurement of radical-sensitive dye. Cell viability was determined on different cell cultures which were exposed to γ-Fe_2O_3@SiO_2-Gl nanoparticles with or without electrostatically bound AsA.

Results: Average size of starting γ-Fe_2O_3 magnetic cores was 13 nm. After coating with SiO_2 and glucose attachment, the diameter increased up to 20 nm; particle size distribution was moderately broad and the particles were stable aqueous colloids. γ-Fe_2O_3@SiO_2-Gl-AsA nanoparticles had up to 70 % of antioxidant activity compared to pure AsA. After incubation with the modified nanoparticles, cytotoxicity assay revealed >90 % cell viability, which proved that the particles were non-toxic.

Discussion and Conclusion: Superparamagnetic γ-Fe_2O_3@SiO_2-Gl-AsA nanoparticles were developed. They were almost non-toxic, colloidally stable in aqueous media, and served as effective scavengers of reactive oxygen species; moreover, they could be easily separated using a magnetic field.

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Neurotoxic level of synthesized, native and physiological nanoparticles: a comparative analysis

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Keywords: nanoparticles, nerve terminals, neurotoxicity

Introduction: Assessment of nanoparticle neurotoxic potential still remains mainly undeveloped. Its importance is underscored by several main factors. On the one hand, nanoparticles are very prospective regarding their use in nanoneuromedicine and neurotheranostics. On the other hand, they are a component of air pollution that is considered to be a potential trigger factor for development of neuropathologies. Unpredictable neurotoxic features of nanoparticles result from unexpected physical and chemical properties of nanomaterials that differ from those of materials in a bulk form. Also, interaction of nanoparticles with different biomolecules originating from the environment and organisms leads to formation of temporal biocorona at the particle surface.

Methods: spectrofluorimetry, readiolabel assay

Results: Herein, we performed a comparative assessment of the neurotoxicity of different types of nanoparticles using similar methodological approaches. Synthesized carbon nanodots, detonation nanodiamonds, native volcanic ash particles, and physiological ferritin-based nanoparticles were analyzed regarding their effects on the key characteristics of glutamatergic and GABAergic neurotransmission. It was shown that the efficiency of neuroactive nanoparticle effects decreased in the line from carbon nanodots, nanodiamonds, ferritin-based nanoparticles to native volcanic ash particles.

Also, native smoke preparations were synthesized and their effects on the above characteristics were revealed.

Discussion and Conclusion: Therefore, synaptic neurotransmission is sensitive to the influence of definite types of nanoparticles and native smoke preparations. This fact underscores an urgent necessity for developing assessment guidelines for neurosafety at the nanoparticle level.

Source(s) of research support This work was supported by Space Research Program of NAS of Ukraine and HORIZON 2020 ERA-PLANET Strand1.

Ethical Approval: Experiments were carried out in accordance with the European Guidelines and International Laws and Policies (Directive 86/609/EEC); the protocols were approved by the Animal Care and Use Committee of the Palladin Institute of Biochemistry (Protocol from 19/09-2012).

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

Assessment of accumulation of carbon nanodots in rat tissues after intravenous administration

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Keywords: Carbon nanodots; biodistribution; accumulation; intravenous administration

Introduction Carbon dots are a newly discovered class of fluorescent carbon nanosized particles. The combination of fluorescent and neuroactive features of carbon dots (and their nanocomposites) makes them useful for visualization of key transport mechanisms in nerve terminals and in neurotheranostics. Recently, we revealed neuroactive properties of synthesized carbon dots. However, their distribution in organisms and ability to be accumulated in different tissues *in vivo* still remains unclear. Here, we investigated the ability of carbon nanoparticles to accumulate in rat tissues after intravenous tail injection.
Methods Preparative biochemistry, fluorescence and radiolabel assay.
Results Carbon nanodots were synthesized from β-alanine and experiments *in vitro* were carried out to prove their membrane active features using spectrofluorimetry and radiolabeled neurotransmitters. *In vivo* experiments were conducted during the week. Intravenous injection of carbon nanodots was carried out daily, with the concentration of carbon dots of 24 mg and 48 mg per kg of body mass, while the content of carbon dots in blood plasma was measured in an hour and in a day after the nanodot administration. The content of nanodots was assessed on the basis of fluorescence intensity. At the end of the experiment, the animals were sacrificed and the tissue preparations of the heart, kidneys, liver and brain were examined.
Discussion - Conclusion It was shown that at the concentrations applied and duration conditions used, carbon nanodots were not accumulated in examined rat tissues after intravenous administration.

Ethical Committee Approval: Experiments were carried out in accordance with the European Guidelines and International Laws and Policies (Directive 86/609/EEC); the protocols were approved by the Animal Care and Use Committee of the Palladin Institute of Biochemistry (Protocol # 2 from 19/09-2014).

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EXCELLENCE IN STUDENTS RESEARCH

Nimodipine treatment preserves the efficacy of neurovascular coupling in the ischemic rat cerebral cortex

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Keywords: neurovascular coupling, cortical spreading depression; nimodipine; hemodynamic brain response

Introduction: In ischemic brain injury, neurovascular coupling is impaired, which causes inadequate perfusion response to neuronal activity. Nimodipine is a clinically used neuroprotective agent, but its effect on neurovascular coupling is unknown. Furthermore, nimodipine administered systematically to patients causes hypotension, an unfavourable side effect. All considered, we aimed to assess the protective effect of nimodipine on neurovascular coupling, and to develop a method for drug delivery that targets ischemic tissue zones exclusively.
Methods: Two open cranial windows were prepared over the parietal cortex of isoflurane-anesthetized, male Sprague-Dawley rats (n=28). The right femoral artery was cannulated for blood pressure (BP) monitoring. In half of the animals, the common carotid arteries were occluded (2VO). Local field potential and cerebral blood flow (CBF) were recorded from the rostral craniotomy, in which nimodipine (100 μM) or its vehicle were administered topically. CBF was assessed in response to repeated whisker stimulation and subsequent spreading depolarization (SD) events triggered by 1 M KCl applied in the caudal craniotomy.
Results: Nimodipine significantly increased the magnitude of hyperemia in response to whisker stimulation (15.4±6.6 vs. 6.2±2.9%, 2VO nimodipine vs. vehicle), decreased the amplitude of SD (13.0±3.0 vs. 15.5±2.6 mV, 2VO nimodipine vs. vehicle), and increased the amplitude of hyperpolarization subsequent to SD (3.7±0.6 vs. 2.2±0.9 mV, 2VO nimodipine vs. vehicle). At the same time, nimodipine excerted no impact on BP (107.5±18.9 vs. 111.3±13.7%, intact nimodipine vs. vehicle), but elevated baseline CBF gradually (120.1±16.8 vs. 102.4±1.9%, intact nimodipine vs. vehicle).
Discussion and conclusion: Nimodipine effectively protects neurovascular coupling in the ischemic brain, and inhibits the evolution of injurious SD. Since local nimodipine administration did not alter BP in our experiments, our next project will focus on intravenous targeted drug delivery with nanoparticles that have been designed to release nimodipine in response to ischemic tissue acidosis.

Ethical Committee or Institutional Animal Care and Use Committee Approval: The experimental procedures were approved by the National Food Chain Safety and Animal Health Directorate of Csongrád County, Hungary (Licence number: XXXII./878/2015). The procedures conformed to the guidelines of the Scientific Committee of Animal Experimentation of the Hungarian Academy of Sciences (updated Law and Regulations on Animal Protection: 40/2013. (II. 14.) Gov. of Hungary), following the EU Directive 2010/63/EU on the protection of animals used for scientific purposes.
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Assessment of the small intestinal blood flow by indocyanine green fluorescence using color-fluorescence laparoscope

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Keywords: indocyanine green, fluorescence, ischemia, blood flow, intestine

Introduction: Indocyanine green (ICG) is a sterile, water-soluble compound that can be administered intravenously or intra-arterially. ICG has a fluorescence property, emitting near-infrared (NIR) fluorescence with 800-850nm wavelength. We can assess blood and lymphatic flow by using a fluorescence camera. ICG is rapidly and extensively bound to protein (mainly albumin) and is confined to the intravascular compartment with minimal leakage into interstitial tissues. Almost 100% of ICG is cleared by liver, and then excreted into bile.
We assessed the presence of reperfusion of ischemic small bowels with ICG. In general, reperfusion of ischemic small bowel is ceased in about 6 hours and its irreversibility determines the surgical indication. Therefore, the aim of the present study is to compare the difference of ICG fluorescence (i.e. blood perfusion) between 4 and 7 hours after ischemia has been induced.
Methods: Four swine were anesthetized and underwent laparoscopy. Two parts of small intestine were used on each swine (i.e. 8 parts were used in total). The blood flow of each part of small intestine was clamped to induce ischemia, one part for 4 hours and the other part for 7 hours. ICG (1mg/kg) was injected intravenously into each of them before de-clamping and blood flow was observed. The prototype of color-fluorescent laparoscope equipped with Hyper-Eye CCD was used for detecting ICG fluorescence.
Results: Fluorescence of ICG along with reperfusion blood flow after 4 hours of clamping were identified in all swine. On the other hand, the ones after 7 hours of clamping did not show reperfusion.
Discussion and Conclusion: These results were consistent with the general fact that ischemic necrosis starts around 6 hours after bowel ischemia is induced. The difference of blood flow between clamping for 4 hours and 7 hours was shown accordingly by the ICG method using a color-fluorescence laparoscope.

Ethical Committee or Institutional Animal Care and Use Committee Approval:
Ethics committee, International University of Health and Welfare Mita Hospital. Date: May 19th, 2010, Approval number: H20-22

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Nanofabricated poly(vinyl alcohol) scaffolds for abdominal hernia repair

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Keywords: Abdominal Hernia, Surgical Mesh, Tissue Engineering, Electrospinning

Introduction: Abdominal hernia is defined as any organ or tissue protrusion through a defect in the abdominal muscular wall, therefore, the only definitive treatment is surgery. Widely popular is the mesh repair involving implantation of a surgical prosthetic mesh to repair the defect, strengthen the abdominal wall and prevent recurrence. It is observed however, that currently applied dry, woven, non-absorbable meshes are far from perfect. In this regard our objective was the production of a biocompatible mesh as a possible alternative to the presently utilized ones.
Methods: Nanofabricated poly(vinyl alcohol) (PVA) meshes were electrospun with a home-made instrument (15 kV potential, 15 cm target distance, 1 mm/h flow rate). Post-electrospinning processing was implemented to strengthen the scaffolds and subsequent sterilization was performed using ClO_2. *In vivo* studies began with a preliminary biocompatibility study on Wistar rats (Group I, n: 15) where scaffolds were implanted intraperitoneally; then continued with two more groups (Group II, Group III, n: 30), where artificial defects (D: 2 cm) were created and thereupon repaired with PVA scaffolds (D: 2.5 cm). A fourth group (Group IV) served as control where the defects were repaired conventionally. A preliminary study with larger animals was then performed on pigs (n: 2). PVA and polypropylene meshes (D: 8 cm) were implanted intraperitoneally and fixed on each side of the anterior abdominal wall. Rats from each group were terminated on the 7th, 14th, 28th, 90th and 180th postoperative days while the pigs during the 5th week. Scaffolds were evaluated macroscopically and microscopically.
Results: No complications were observed in any of the animals. All animals survived until their termination date while histological studies revealed physiological wound healing and scaffold integration.
Discussion: The results are positive but further long-term studies are required.
Conclusion: PVA meshes indeed have potential future prospects as alternative surgical meshes.

Ethical Committee or Institutional Animal Care and Use Committee Approval: The experimental protocol adhered to rules laid down by the Directive of the European Parliament and of the Council on the protection of animals used for scientific purposes, and was approved by the Semmelweis University's Institutional Animal Care and Use Committee. The accreditation number of the laboratory is 22.1/1244/3/2011.
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The rapid effect of 17-β-estradiol on diffusion dynamics of p75 receptor in live neurons

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Keywords: estradiol, p75 neurotrophin receptor, single molecule imaging, non-classical action

Introduction: In addition to classical genomic action, gonadal steroid 17-β-estradiol (E2) is known to exert rapid non-classical effects on membrane receptors and signaling molecules. The changes in surface movement of the receptors are essential to their function. These changes form membrane protein complexes that activate signalling molecules. Neurotrophin receptor p75 (NTR) is a regulator of neuronal survival. The key mechanism in NTR activation is the change of surface movement of the receptor. However, the effect of E2 on NTR surface trafficking is completely unknown.
Methods: We applied a unique total internal reflection microscopy system to allow super-resolution imaging of membrane receptor molecule surface trafficking. Trajectories of NTR molecules on live neurons were individually tracked and analysed. Mean square displacement (MSD) and the diffusion coefficient (D: μm2/sec) were determined both on the soma and neurites. Movement parameters were calculated using at least 200 trajectories and compared statistically to the corresponding vehicle control.
Results: The MSD function of NTR molecules in the soma and neurites saturates, suggesting restricted motion of NTR molecules in control conditions. After 100nM E2 application the MSD curve of NTR molecules changes to a straight line in soma and neurites demonstrating unrestricted motion of NTR molecules after E2 application. Furthermore, our results showed that administration of 100 nM E2 rapidly increases the D of NTR molecules both on the soma and neurites.
Discussion: The MSD function and D of NTR molecules showed that surface movement of NTR molecules changed to unrestricted after 100 nM E2 application and E2 rapidly increased the surface diffusion of NTR molecules. These effects were irrespective of the location of NTR in the neuronal membrane.
Conclusion: Our findings demonstrated that E2 rapidly alters the surface diffusion of NTR molecules. The effect of E2 on molecule movement of NTR may be implicated as a neuroprotective mechanism of E2.

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Sexual differences in the biomechanics and vasoreactivity of coronary resistance arteries in exercise induced left ventricular hypertrophy

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Keywords: coronary adaptation, gender differences

Introduction: There is evidence that sustained, heavy physical exercise has effects on the heart, and it is also well known that the sex influences the cardiovascular risk. Most studies are based on the epicardial coronary arteries; common knowledge is still lacking about the functional adaptations of small arterioles. Synthesizing this evidence and changing the region of interest, in this study we observed the different ways of adaptations of resistance coronary arterioles in left ventricular (LV) hypertrophy depending on gender.
Methods: Young adult Wistar rats were distributed into four groups with 8 rats in each group. They were separated by the sex and half of the groups were trained by a 12 weeks long heavy swimming exercise program lasting 200 min/day. The controls also spent time in the water for 5 min/day. Ventricular adaptation was controlled by echocardiography at 11 weeks. At the termination of the study the intramural resistance coronary arteries (200 μm outer diameter) were removed to examine the biomechanical adaptation by pressure arteriography. Contractility, endothelium dependent dilation (EDD), tangential wall stress and elastic modulus were examined. Elastic remodeling was studied on resorcin-fuchsin stained histological sections.
Results: Relative heart mass increased in swimmers without arterial hypertension ($p<0.001$), ejection fraction ($p<0.001$) and fractional shortening were both elevated ($p<0.001$). Resistance arteries had thicker walls and reduced isobaric tangential wall stress ($p<0.05$). Elastic modulus at physiological pressures and density of inner elastic membrane increased in swimmers ($p<0.05$). Both spontaneous ($p<0.05$) and TxA_2 agonist induced tone ($p<0.001$) were elevated and endothelium dependent (bradykinin, $p<0.05$) and independent (adenosine, $p<0.001$) relaxations were more effective. Female swimmers had more vigorous contraction ($p<0.001$), while male swimmers had a more improved endothelial dependent vasodilation ($p<0.025$).
Discussion: Our results contribute to the understanding of sexual differences in coronary adaptation.
Conclusion: The range of vascular reactivity in coronary segments increased in both genders, but its mechanism was different between males and females.

Institutional Animal Care Committee Approval: Allatkiserleti Tudomanyos Etikai Tanacs (ATET) Animal Research Ethics Committee Permit on January 27, 2015 permit number KA 1661, Reference number: PEI/001/802-2/2015
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LIFE SCIENCES PROGRESS IN UKRAINE REPORTED IN THE UKRAINIAN BIOCHEMICAL JOURNAL

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Fatty acid composition of phospholipids of adipocytes in different age rats with obesity-induced insulin resistance and the effect of N-stearoylethanolamine

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Keywords: obesity, insulin resistance, adipose tissue, dyslipidemia, N-Stearoylethanolamine

Introduction: Chronic hypernutrition and high fat diet (HFD) rich in saturated fatty acids leads to molecular changes in insulin sensitive tissues and is followed by dyslipidemia. Phospholipids (PL), as one of the main components of cell membranes, are involved in mechanisms of insulin signaling. That is why the aim of our study was to investigate the fatty acid (FA) composition of PL of adipocytes in different age rats with HFD-induced insulin resistance (IR) and its changes under N-stearoylethanolamine (NSE) administration.
Methods: The experimental model was induced in rats at ages 10 months and 24 months by HFD and confirmed by the oral glucose tolerance test and HOMA-IR. NSE was administrated *per os* for 2 weeks. Adipocytes were isolated from abdominal fat using type 1 collagenase solution. Adipocyte lipid extract was separated into the fractions by thin-layer chromatography. Phospholipid fatty acid composition was analyzed by gas-liquid chromatography.
Results: The investigation of the FA composition of PL demonstrated that total content of FA is significantly higher in control and IR groups of 10-month old rats compared to the same groups of older animals and NSE normalized FA content in adipocyte PL of the older rats. In both age groups after HFD we observed a statistically significant growth of saturated fatty acids (SFA) as well as unsaturated (UFA). There was no significant difference in the ratio of SFA:UFA between control animals at different ages but HFD induced a considerable decrease of FA saturation in PL of adipocytes of older animals.
Discussion and Conclusions: It was demonstrated that prolonged HFD induced IR and leads to changes in the FA profile of adipocyte PL in rats from two age groups. The results support an impact of imbalance in FA of adipocyte PL on impairment of insulin sensitivity. As far as NSE administration had a positive effect on normalization of FA composition of PL in adipocytes, we can consider NSE as a prospective agent for the treatment of obesity-induced complications and correction of age-related dyslipidemia.

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Ethical approval: All experiments involving animals were carried out with the approval of the Animal Care and Use Committee of the Palladin Institute of Biochemistry, National Academy of Sciences of Ukraine (Protocol №1 from 08/09-2015).
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Clot formation and lysis in platelet rich plasma of healthy donors and patients with resistant hypertension

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Keywords: platelets, clotting, lysis, clot waveform analysis, arterial hypertension

Introduction: Hemostatic balance in blood is affected by numerous factors, including coagulation and fibrinolytic proteins, the wide spectrum of their inhibitors, and blood cells. Since platelets can participate in contradictory processes, they significantly complicate the whole hemostasis picture. Therefore, nowadays the development of global assays of hemostasis, which can reflect the physiological process of hemostasis and can be used for point-of-care diagnosisof thrombosis, is crucial.
Methods: The clot waveform analysis (CWA) was used to study the integral effect of platelets on the evolution of the fibrin clot, caused by the combined effect of coagulation and fibrinolytic factors (8 mM $CaCl_2$, 0.5 nM thrombin and 15 or 40 IU/ml rt-PA), and to analyze CWA capability to distinguish the response of platelet-rich plasma (PRP) of healthy donors and patients with arterial hypertension. Formation and lysis of clots obtained from PRP ($300x10^3$ platelets/μl) were monitored by measurements of plasma absorbance changes at 405 nm.
Results: When coagulation was initiated with $CaCl_2$ (to activate the intrinsic coagulation pathway) or with $CaCl_2$ and thrombin (to establish the influence of platelets on terminal reactions of coagulation cascade), platelets stimulated coagulation in direct proportion to the cell number. It was found that the clotting/lysis parameters of donor PRP only moderately altered with the addition of 15 IU/ml rt-PA; under the influence of 40 IU/ml rt-PA platelets potentiated fibrinolysis more than coagulation. At the same time, for patients with hypertension, platelets, embedded in the PRP-derived clot, showed a weaker ability to stimulate fibrinolysis, especially poorly promoting the lysis by 15 IU/ml rt-PA; this indicates that the exogenous activator does not stimulate the profibrinolytic properties of platelets in patients as much as it was shown for donors.
Discussion and Conclusion: The obtained data give evidence that platelets can act not only as procoagulants but also as profibrinolytics. By simultaneously amplifying coagulation and fibrinolysis, making their rates comparable, platelets would control plasma procoagulant activity, thereby regulating local hemostatic balance, the size and lifetime of the clot. Moreover, CWA may be used to distinguish the PRP response in CWA from healthy donors and patients with essential hypertension.

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Ethical Approval: Studies were approved by the local research ethics committees of Palladin Institute of Biochemistry of the National Academy of Sciences of Ukraine (№3, from 30.08.2017). All participants gave written informed consent.
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Osteoprotective effects of vitamin D_3 in diabetic mice is VDR-mediated and regulated via RANKL/RANK/OPG axis

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Keywords: Vitamin D_3, diabetes, secondary osteoporosis, VDR, RANKL.

Introduction: Vitamin D_3 deficiency is known to be associated with the development of low bone mineral density related to primary osteoporosis and other disorders, including diabetes. The present study was designed to determine the relationship between vitamin D_3 availability, vitamin D receptor (VDR) expression and the RANKL/RANK/NF-κB signaling pathway in the activation of bone resorption and loss of bone architecture associated with type 1 diabetes.
Methods: Diabetes was induced in male C57BL/J6 mice by i.p. injection of multiple low dose streptozotocin (40 mg/kg b.w.). Control and diabetic mice were treated with or without vitamin D_3 (15 IU/mouse per os, for 8 weeks). Serum 25-hydroxyvitamin D_3 (25OHD) was assessed by ELISA. The protein levels of VDR, RANKL, RANK, osteoprotegerin (OPG), phosph. NF-κB/p65 and osteocalcin (OC) in bone tissue were assayed by WB. The biomechanical properties were analyzed with the 3-point bending test. μCT analysis was performed on the proximal tibia by using Skyscan 1072.
Results: Serum level of 25OHD was shown to be reduced to 22.5±1.8 in diabetes vs. 41.7±2.8 nmol/l in control, that reliably reflects the vitamin D_3 deficiency ($p<0.05$). These changes were accompanied by significant changes in bone biomechanical properties. Diabetes caused a decrease in maximum load, toughness and stiffness of diabetic tibias by 2.1-, 3.8- and 2.3-fold respectively ($p<0.05$). Histomorphometry of tibias showed respectively 3.0-, 2.1- and 1.3-fold decreases in bone volume per tissue volume (BV/TV), trabecular number (Tb.N) and cortical thickness (Cort.Th) in diabetes vs. control ($p<0.05$). Diabetes led to up-regulation of phosho-p65, RANKL, RANK (2.3-, 1.72-, 1.51- fold respectively) and down-regulation of OC, OPG and VDR (1.5-, 1.6- and 1.8-fold respectively) in bone tissue of diabetic mice ($p<0.05$). Treatment with vitamin D_3 improved diabetes-induced structural and biomechanical abnormalities in bone tissue.
Conclusion: The findings suggest a significant role of RANKL/RANK/NF-κB signaling pathway activation in the induction of bone resorption in diabetes that occurs on the background of vitamin D_3 deficiency. It was demonstrated that diabetes-related impairments may efficiently be corrected by vitamin D_3 treatment.

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Ethical Committee Approval: Institutional Animal Care and Use Committee Approval: PIB 05/01/2016 No 1.

Evaluation of antiproliferative activity of novel pyrazolothiazolopyrimidine derivatives

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Keywords: pyrazolothiazolopyrimidines, Doxorubicin, antiproliferative activity.

Introduction: Heterocyclic pyrazolopyridine-based derivatives are attractive for pharmaceutical and medicinal chemists who design and synthesize new anticancer agents.
Methods: The anti-proliferative activities of seven novel 8-methyl-2-R-7-[R`-phenilmethyl]pyrazolo[4,3-e][1,3]thiazolo[3,2-a]pyrimidin-4(2H)-one derivative compounds were evaluated using MTT testing of human tumor cell lines of different tissue origin (leukemia (K-562, HL-60, HL-60/ADR), melanoma (SK-MEL-28), glioblastoma (U-251 MG), colon (HCT116), hepatocyte (HepG2), ovary (SKOV3) and breast (MCF-7)), as well as of non-tumor cells (HEK293, HaCaT and J774.2).
Results: It was demonstrated that cytotoxic effects of these derivatives depended on the tissue origin of targeted cells. Leukemia K-562 and HL-60 cells were the most sensitive to the action of compounds 2 and 7. Compound 2 demonstrated approximately two times higher toxicity towards the multidrug-resistant sub-line of HL-60/ADR cells when compared with the action of doxorubicin. Human breast and ovarian carcinoma cells demonstrated the weakest sensitivity to the pyrazolothiazolopyrimidine derivatives. These derivatives were less toxic than doxorubicin towards non-tumor HEK293, HaCaT and J774.2 cells.
Discussion: Antiproliferative action of compounds 2 and 7 dropped as follows: leukemia > melanoma > hepatocarcinoma > glioblastoma > colon carcinoma > breast and ovarian carcinoma cells. These compounds were less toxic than doxorubicin towards the non-tumor cells.
Conclusion: The novel pyrazolothiazolopyrimidine, compound 2, demonstrated high toxicity towards human leukemia and, of special importance, towards the multidrug-resistant leukemia cells. Their low toxicity towards pseudo-normal cells was found.

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Impact of N-acetylcysteine on antitumor activity of doxorubicin and landomycin A in NK/Ly lymphoma-bearing mice

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Key words: N-acetylcysteine, doxorubicin, landomycin A, NK/Ly lymphoma.

Introduction. The problem with many antitumor drugs is their side effects, because they affect both malignant and normal cells of an organism. So, novel approaches should be developed to decrease side effects of anticancer drugs and enhance their therapeutic effect. The aim of this study was to investigate the impact of N-acetylcysteine (NAC) on antitumor activity of the traditional anticancer agent doxorubicin (Dx) and of the experimental agent landomycin A (LA) towards NK/Ly lymphoma-bearing mice.
Methods. To investigate this impact, survival time and weight changes of NK/Ly lymphoma-bearing animals treated with Dx or LA in combination with NAC were carried out by daily weighing of experimental animals and control of their physiological state. The hematological profile of animals was studied by the analysis of blood smears under light microscopy. Nephrotoxicity of the studied drugs was evaluated by measuring the activity of creatinine.
Results. It has been revealed that NAC significantly decreased nephrotoxicity of Dx, possessed moderate immunomodulating activity and also partially increased survival of NK/Ly lymphoma bearing animals under Dx treatment. On contrary, there was little tissue-protective effect of NAC towards LA due to weak side effects of this anticancer drug, however, combined used of NAC and LA significantly increased survival (60+ days) of LA-treated animals with NK/Ly lymphoma.
Discussion and Conclusion. NAC reduces NK/Ly lymphoma-induced neutrophilia and lymphopenia under Dx treatment, as well as decreases monocytosis, caused by Dx in tumor-bearing animals. Additionally, this antioxidant partially reverses nephrotoxicity of Dx, thus leading to increased survival of tumor-bearing animals under Dx treatment. Thus, the observed immunomodulating and kidney-protecting properties of NAC may be of high importance for lowering side effects of Dx in clinical practice. On the contrary, NAC had no effect on the blood profile of animals treated with LA due to low toxicity of this experimental drug, but partially reversed LA-induced nephrotoxicity and led to complete remission in mice with NK/Ly lymphoma, treated with LA and NAC. Studies of molecular mechanisms underlying such specific features of NAC are in progress. All *in vivo* experiments were conducted in accordance with the international principles of the European Convention for protection of vertebrate animals under control of the Bio-Ethics Committee of the above mentioned institution (Protocol № 4/2017 from 1.06.2017 of the BioEthics Committee at the Institute of Cell Biology, NAS of Ukraine).
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Research and development of peritoneal dialysis solutions

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Keywords: peritoneal dialysis, viability, glucose degradation products, MTT

Introduction: Peritoneal dialysis (PD) is an essential modality of renal replacement therapy. Currently, there are no convincing conclusions about the benefits to patients of solutions with a low content of glucose degradation products. A detailed review of the literature data concerning PD solutions revealed that there are needs for their pharmaceutical development.
Methods: The following research methods were used: argentometry, spectrophotometry, potentiometry, cell viability *in vitro*, methods of statistical processing of results.
Results: Research findings of the less expensive traditional PD solutions are presented. The following studies were performed: elaborating rapid procedures for determination of chloride ions by argentometry and the 3,4-dideoxyglucoson-3-en (3,4-DGE) and 5-hydroxymethylfurfural (5-HMF) content by spectrophotometry, viability of Vero line cells in the presence of the developed PD solutions by means of neutral red (NR), MTT and sulforhodamin B (SRB) and establishing correlations between the cell viability and analytical indexes. The weak but expected correlation coefficient (0.31) was established in the MTT test between the viability growth and an increase in the pH of the PD solutions.
Discussion: The results of the study confirm the cytotoxicity of the PD solutions. However, because it is not yet known what factors or their combination cause cytotoxicity, further complex studies are needed. The highest cytotoxicity was detected in the MTT test, the lowest one was in the SRB test, indicating the largest mitochondrial vulnerability to the influence of PD solutions. The NR test is not suitable for comparative studies of PD solutions which differ in pH, as it does not enable the comparison of plausible cell viability after increasing pH solutions and/or improving their manufacture.
Conclusion: These research findings serve as a foundation for development of PD solutions and could be useful in the context of planning the manufacture of PD solutions in cost-conscious countries, including Ukraine.

Acknowledgement: We thank Cedars-Sinai Medical Center's International Research and Innovation in Medicine Program, the Association for Regional Cooperation in the Fields of Health, Science and Technology (RECOOP HST Association) for their support of our organization as participating Cedars-Sinai Medical Center-RECOOP Research Centers (CRRS). Nataliia Hudz thanks the International Visegrad Fund (contract No. 51700107) for providing scholarship for studies related to solutions for dialysis therapy.
Ethical Committee or Institutional Animal Care and Use Committee Approval: DHLNMU 22/01/2007 № 1

Simple two-step covalent protein conjugation to PEG-coated nanocrystals

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Key words: PEGylated nanocomposites, quantum dots, covalent protein conjugation.

Introduction: Covering of nanocrystals (NC) with a polyethylene glycol (PEG) envelope is a common way to increase their hydrophilicity, and compatibility with bio-systems, including increased retention time in the body. Colloidal semiconductor NC, also known as quantum dots (QD), particularly benefit from covering with PEG due to passivation of the inorganic core, while maintaining physical properties of the core. Despite many advantages of covering the surface with PEG, the covalent attachment of protein to hydroxyls of PEG is complicated.
Methods: NC with a PEG envelope were modified with monochloroacetic acid. The obtained carboxy-PEG-NC entered the reaction of a covalent protein attachment using zero-length cross-linker 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride. Fluorescent microscopy was performed with a Carl Zeiss AxioImager A1 DIC/fluorescent microscope (Oberkochen, Germany) using a 1.3 NA 100× oil immersion objective. Fluorescent images were taken by a Zeiss AxioCam MRm III cooled digital CCD camera under constant exposure. Fluorescence of NC was evaluated at red channel (610 nm emission was obtained at 532 nm excitation). Image analysis was performed using Fiji software (National Institutes of Health [NIH], Bethesda, MD, USA).
Results and Discussion: Here we propose a simple two-step approach for modification of PEG residues with subsequent covalent attachment of proteins. We were able to achieve specific NC targeting by means of attached protein as well as preserve their optical parameters (fluorescence intensity) in chemical reaction conditions. In the optimized protocol, ensuring removal of chemical byproducts by dialysis, we were able to omit the need for centrifugation (usually a limiting step due to particle size). The obtained NC-protein conjugate solutions contained 0.25x of the initial unmodified NC amount, ensuring a low dilution of the sample. During all reactions the pH range was optimized to be between 6 to 8.
Conclusion: The proposed approach can be easily modified for covalent targeting of different PEG-covered nanocomposites with proteins.

Source(s) of research support: The study was supported by Grants from the Ministry of Healthcare of Ukraine (project 0116U000759) and the Sonata 8 project no. UMO-2014/15/D/ST5/02744.

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

RECOOP RESEARCH IN PROGRESS
Part II

Interactive Poster Presentations

Sex differences of metformin effects on metabolic risk factors in patients with metabolic syndrome.

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Keywords: metabolic syndrome, sex difference, metformin.

Introduction: Metabolic syndrome is one of the major health and development challenges of the XXI century. The problem of "civilization disease" is determined not only by considerable spread, but also by the progressive tendency towards "rejuvenation". Metformin is the drug of choice to relieve the main risk factors of metabolic syndrome development.
Material and methods: The study included 60 patients (30 men and 30 women), who underwent treatment in Lviv Municipal Clinical First Aid Hospital. Mean age of patients was 50,1±5,8 years. All patients were divided in two groups depending on gender variability. Each group took metformin in a 1500 mg dose. Blood pressure, blood glucose, HbA1c, insulin resistance indices, lipid profile and anthropometric parameters (body mass index, waist circumference, the circumference of the hips and their ratio) were measured for all patients prior to and after 6-month treatment. Diagnosis of metabolic syndrome was confirmed according to criteria IDF-2015.
Results: The results of the study showed that there was no difference in HbA1c between men (before treatment 7,9±0,95%, after 6,8±0,8%, $p<0,05$) and women (before 7,7±0,5% after 6,2±0,7%, $p<0,05$), and in HDL (men before treatment 1,0±0,1mmol/l, after treatment 1,0±0,2mmol/l, $p>0,05$; women before treatment 0,8±0,1mmol/l, after treatment 0,8±0,1mmol/l, $p>0,05$). However, there was a significant difference in decrease of total cholesterol between men (before treatment 6,1±1,2 mmol/l, after treatment 5,9±1,2mmol/l, $p>0,05$;) and women (before treatment 5,5±1,3mmol/l, after treatment 4,2±0,9mmol/l, $p<0,05$). Also a difference in LDL in men (before treatment 4,2±1,0mmol/l, after 4,1±1,0 mmol/l, $p>0,05$) and women (before 3,0±1,4mmol/l, after 1,9±1,6 mmol/l, $p<0,05$) was found.
Discussion: Metformin improves the glycemic profile, antropometric parametres, and some parametres of lipid profile both in men and women, with no significant differences.
Conclusions: The metformin effect on LDL and total cholesterol was more noticeable in women than in men. Further research is required to find the root cause of this effect.

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Ethical Committee or Institutional Animal Care and Use Committee Approval
Danylo Halytsky Lviv National Medical University, 15/02/2015 №2

The expression of the connexin 37 gene in the aorta of rat models of dyslipidemia, hypertension and dicarbonyl stress

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Key words: connexin 37 gene, dyslipidemia, hypertension, aorta, experimental model

Introduction: Connexin 37 (Cx37) is transmembrane protein that forms intercellular gap junction channels. Cx37 participates substantially in the communication between cells within the vascular wall. Association studies have revealed polymorphism of the Cx37 gene as a possible risk factor for atherosclerosis. We tested the expression of Cx37 in the aorta of rat models of dyslipidemia, hypertension and dicarbonyl stress to obtain information about the possible role of Cx37 in the pathogenesis of vascular illnesses.
Methods: Methylglyoxal (MG), a key player in the development of vascular complications of diabetes, was intragastrically administered (3 times a week, 0.5 mg/kg body weight, four weeks) to hereditary hypertriglyceridemic rats (HHTg), an animal model of metabolic syndrome and insulin resistance. The control group consisted of HHTg rats without MG. The effect of hypertension and dicarbonyl stress was studied in spontaneously hypertensive rats with decreased expression of glyoxalase 1 (SHR-Glo1$^{+/-}$) which detoxifies MG, and in SHR controls. Relative expression of the Cx37 gene was assessed in the supra- and infrarenal abdominal aorta in 6 HHTG and in 3 PHC (Prague hereditary hypercholesterolemic rat) females. Relative expression of Cx37 mRNA was measured by real time PCR. Data are expressed as mean±SE and were tested by unpaired t-test.
Results: Expression of Cx37 in HHTg rats after MG administration was not significantly reduced compared to controls (0.755±0.101 vs. 1.042±0.117, p=0.092). In contrast, in the SHR-Glo1$^{+/-}$ model, the expression was significantly higher than in controls (1.940±0.348 vs. 1.028±0.083, p=0.032). The expression was also significantly lower in the supra- than in the infrarenal segment of aorta in the HHTg group (1.191±0.338 vs. 2.712±0.419, p=0.019) and PHC group (1.178±0.315 vs. 5.101±1.167, p=0.069).
Discussion and Conclusion: The results show a possible relationship between dicarbonyl stress and the expression of Cx37 in the context of dyslipidemia and hypertension. Expression of the Cx37 gene substantially differs between the supra- and infrarenal abdominal aorta.

Source of research: Supported by MH CZ - DRO ("Institute for Clinical and Experimental Medicine - IKEM, IN 00023001“).
Ethical Committee Approval: All of the experiments were performed in agreement with the Animal Protection Law of the Czech Republic (311/1997) and approved by the Ethics Committee of the Institute for Clinical and Experimental Medicine MZ 43/2014.
Acknowledgement: We thank Cedars - Sinai Medical Center’s International Research and Innovation in Medicine Program, the Association for Regional Cooperation in the Fields of Health, Science and Technology (RECOOP HST Association) for their support of our organization as participating Cedars – Sinai Medical Center - RECOOP Research Centers (CRRC).

Ultrasonographic aspect of glycemic control of placental vascularization in type 1 diabetes mellitus

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Keywords: glycemic control; placental vascularization; type 1 diabetes mellitus; three-dimensional power Doppler indices; ultrasound

Introduction: The aim of our study was to assess glycemic control by placental vascularization in pregnancies complicated by type 1 diabetes mellitus (T1DM) and to compare the dataset to normal placental 3-dimensional power Doppler (3-DPD) indices.
Methods: Placental vascularization of pregnant women was prospectively evaluated by 3-DPD ((vascularization-index (VI); flow-index (FI); vascularization-flow-index (VFI)) ultrasound technique. The normal pregnancies (n=214) were compared to those complicated by T1DM (n=53) with optimal (HgA1c≤6%;≤42mmol/mol) and suboptimal (HgA1c>6%; >42mmol/mol) glycemic control.
Results: Pregnancies complicated by T1DM expressed lower placental vascularization indices as compared with normal pregnancies (adjusted odds ratio (AOR) for VI:0.86; FI:0.94; VFI:0.76). Placental 3-DPD indices have significant correlation with HgA1c and optimal glycemic control is associated with lower placental perfusion (AOR for VI:1.64; FI:1.13; VFI:2.34). Short-term adverse neonatal outcome was predicted by lower 3-DPD indices (AOR_{VI}:0.83, AOR_{FI}:0.93, AOR_{VFI}:0.66, $p<0.05$ for each indices). Besides glycemic control, the pre-gestational body mass index, blood pressure and fetal sex had significant influence on placental perfusion.
Discussion: VI displayed the best screening property for suboptimal glycemic control with a sensitivity of 90.9%.
Conclusion: The suboptimal glycemic control has a direct deteriorating effect on placental vasculature. Therefore, the 3-DPD evaulation could be an adjunct diagnostic modality for pregnant women with T1DM.

Ethical Committee Approval: University of Szeged No 135/2011.

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Might Fungal Sinusitis Be Related to Lowered Indoor Air Quality?

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Keywords: Airborne Fungi; Fungal Toxicants; Allergy; Dwellings.

Introduction: Chronic nasal obstruction (CNO), incl. fungal rhinitis and sinusitis (or combined rhinosinusitis), makes breathing difficult or even impossible. The aim of this study was to clarify the relationship between chronic rhinitis incidence and indoor mycological quality of patient dwellings in Slovakia for the first time.
Materials/Methods: Over a 7-yr period, ORL biological specimens from 69 patients with polyposis (surgically removed polyps), allergic rhinitis (swabs, mucous) and 20 healthy controls (swabs) underwent complex myco-lab analysis. Extended objective mycoanalyses of the patients' houses were performed after their epid-study focused on indoor fungi. In the end, the relation between enviro-fungi and occupational fungal CNO under Slovak housing conditions was characterized statistically.
Results: At least one fungus in all groups of probands tested was isolated from biological specimens. For the data from 39 individuals (29 patients, 10 controls) statistical analysis was performed. Statistically significant higher fungal isolate counts in the biological specimens, $p<0.05$, was found in controls. Qualitative and quantitative mycobiotic composition of indoor and related outdoor environments of the dwellings of the controls was similar: the primary, incl. *Aspergillus versicolor*, and secondary colonizers dominated, but the tertiary ones were sparse.
Discussion: There was no statistically relevant difference found in fungal colonization of nasal mucous between healthy individuals and patients suffering from chronic rhinosinusitis, similar to findings of some US studies. The airborne indoor mycobiota proved to be a statistically important colonizer of the patient nasal cavity. As toxic mold *Aspergillus versicolor* was detected in the indoor environments, the affected ones must be considered as hygienically risky.
Conclusion: We favor the opinion that non-invasive non-pathogenic fungi naturally colonize the mucus of the upper airways and they are not the primary causative agent of CNO in the sense of inflammation and/or typical allergic reaction. Reduction of indoor fungal load (incl. propagule burst) showed again to be a powerful factor for minimizing the (chronic) damage of upper airways in occupants.

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Gender difference in glucocorticoid, insulin and estrogen receptors expression upon chronic stress and aging

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Keywords: stress, aging, neurodegeneration, insulin, estrogen, glucocorticoid receptor

Introduction: Insulin and estrogen signaling have been associated to molecular mechanisms of neurodegeneration. Changes in those signalling pathways as well as loss of memory are expected upon stress and aging. In this study, expression of estrogen (ER), insulin (IR) and glucocorticoid (GR) receptors was analyzed.
Methods: Male and female rats were divided in young and old animal groups. Chronic stress protocol was performed for 10 weeks. Expression of ER-α, IR-β and GR was screened by Western-blotting in hippocampi (HIP) and cerebella (CER). The Statistica software was used for data analysis.
Results: Chronic stress caused a decrease of GR in CER of all animal groups, no changes in HIPP of all male groups and a decrease of GR in HIP of all female groups. ER-α expression was increased in CER of all animals with statistical significance reached in case of young males ($p=0.049$). ER-α in HIP increased in all animal groups except for old males. IR-β expression in CER increased in young males and old females and decreased in old males and young females upon stress. IR-β in HIP increased in all young animals upon stress.
Discussion and Conclusion: GR is a transcription factor which adapts the power of stress response. When decreased as in stressed animals, glucose levels in blood decrease and cause low metabolism rate in all cells which is crucial for neuronal survival as well. Estrogen provides neuroprotection and the study shows young males are most affected by ER changes caused by stress. Increased insulin increases the insulin sensitivity and the animals cope better with stress. If IR is decreased, as in all old animals in HIPP, it could be a sign of decompensational mechanisms that leads to neurodegeneration. Previously we observed memory impairment in old females which can be connected to these signalling pathways.
Acknowledgments: This study has been funded by Croatian Science Foundation (IP-09-2014-2324) and Cedars Sinai Medical Center's International Research and Innovation in Medicine Program and the RECOOP HST Association. Part of this study was performed at the Department of Pharmacodynamics and Biopharmacy, Faculty of Pharmacy, University of Szeged, under the supervision of prof. Robert Gaspar.
Ethical Committee Approval: Experiments were carried out at Animal Facility of Faculty of Medicine Osijek and was approved under class number 602-04/14-08/06 and registration number 2158-61-07-14-118 and Animal Facility of Faculty of Pharmacy, Szeged, approval number: CSI/01/3796-7/2015.

The common effect of early transient hyperglycaemia and hyperoxia on the retina and kidney

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Keywords: hyperglycaemia, retinopathy, premature, hyperoxia, kidney

Introduction: Recent studies suggest strong correlation between high glucose ambience and impaired kidney and retinal function. Premature infants often develop transient hyperglycemia and experience alternating hyper/hypoxia. We aim to study the pathological changes of kidney and retina in an animal model under these conditions.
Methods: Newborn Sprague-Dawley rats were cross-fostered and divided into two groups after birth. They were maintained in either normoxic (Cont) or a daily alternating hypo/hyperoxic (HO) environment from postnatal day (PD) 1 to 14. Both groups were further subdivided into two. The pups were injected intraperitoneally with either 100 mg/kg Streptozotocin (STZ) to induce hyperglycemia (HG) or citrate buffer as a control (Cont). On PD17 kidneys and retinas were removed after euthanasia and proceeded to routine HE procedure or immunohistochemistry respectively. Morphological analyses were executed on both organs. Cytokine expression of retinas of the four group were measured by a semi-quantitative method.
Results: STZ treatment resulted in hyperglycemia by PD2 and lasted until PD7 in HO group and PD4 in the controls. The thickness of the Bowman's space was significantly smaller in the HG-Ox group than in the other 3 groups. We observed a difference in the HG-Cont group too compared to controls. The diameters of the proximal tubules were significantly bigger in both of the hyperglycemic groups compared to controls. Cortical thickness was significantly less in both Ox groups than in the normoxic controls. The width of the nephrogenic zone was extended in the HG-Ox group compared to the other three groups. In HO groups ROP developed but hyperglycemia had no influence on the extent of retinopathy, but it disturbed the balance of cytokines.
Discussion and Conclusion: Our results showed, that the acute postnatal hyperglycemia induce detectable changes in the kidneys which may lead to renal impairment later. Although hyperglycemia did not influence ROP morphology but altered cytokine levels may refer to cellular damage. Thus we find it essential to control blood sugar level and monitor kidney function in preterms.

Ethical Committee Approval: University of Pecs approved protocol No: BA02/2000-15024/2011

Source(s) of research support: TÁMOP 4.2.2. D-15/1/KOMV-2015-0004

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Investigation of prenatal smoke-exposure in chronic retinal hypoperfusion on adult rats

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Keywords: pregnancy, prenatal smoke, fetal hypoxia, neurological deficits

Introduction: Numerous studies indicate that smoking during pregnancy may have harmful effects on the offspring. Prenatal smoke exposure (PSE) may lead to fetal hypoxia and ischemia, which affect brain development, and improve the risk of neurological deficits.
Methods: Wistar rats (n=28) were mated and exposed to whole-body smoke exposure for 2 hours daily from mating until delivery. A closed-chamber manual smoking system and 4 research cigarettes per occasion were used. This model represents a passive smoking animal model. Neurobehavioral development was assessed on newborn rats in the first weeks of age. Then, after growing up, we applied a permanent bilateral common carotid artery occlusion (BCCAO) under isoflurane anesthesia and both carotid arteries were ligated through a midline incision. After two weeks of BCCAO all animals were sacrificed with an overdose of anesthetic and the eyes were processed for histological analysis. We measured the following parameters: the width of the outer and inner nuclear and plexiform layers (ONL, OPL, INL, and IPL) the number of cells/100µm section length in the ganglion cell layer (GCL).
Results: BCCAO resulted in severely reduced thickness of retinal layers as observed two weeks after ligation compared to sham-operated controls. Marks of degeneration with individual variations were visible in all retinal layers. The number of cells in the ganglion cell layer decreased significantly by 48%. PSE itself led to the decrease in cell number of the GCL and extenuated. The effects of BCCAO performed in prenatally smoke-exposed animals resulted in significantly thinner retinal layers and a reduced number of cells in the ganglion cell layer.
Discussion and Conclusion: PSE influenced the development of neurological reflexes and signs in neonatal rats slightly. In this study we were able to show not just functional but histological damages on retinal tissue in adult rats prenatally exposed to tobacco smoke.

Ethical Committee Approval: University of Pécs (Nr BA02/2000-15024/2011) for the ethical use of animals.
Source of research: PTE-MTA “Lendület” Program
Acknowledgement: We thank Cedars Sinai Medical Center’s International Research and Innovation in Medicine Program, the Association for Regional Cooperation in the Fields of Health, Science and Technology (RECOOP HST Association) for their support.

Effects of Olaparib and radiation on cervical cancer cell line

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Keywords: Olaparib, HeLa, cervical cancer, radiation

Introduction: In Hungary, even nowadays cervical carcinoma affects hundreds of women and claims 500 lives annually. There is an urgent need to develop novel therapeutic modalities to increase the effectiveness of the therapy. Poly (ADP-ribose) polymerase (PARP) family of proteins is involved in a number of cellular processes, including programmed cell death and DNA damage repair, therefore the inhibition of PARP is a recently developed strategy for oncological therapy. In present study we investigated the separated antiproliferative effects of the PARP inhibitor Olaparib in different concentrations and the combined effects of Olaparib and radiation on a cervical adenocarcinoma cell line, HeLa (ATCC).
Methods: We investigated the viability of cells after Olaparib treatment in different concentrations (8uM, 4uM, 2uM, 1uM, and 500nM). More than half of cervical cancer patients receive radiotherapy, therefore we examined how Olaparib pretreatment can influence the effectiveness of radiation. We measured cell viability with MTT test and applied radiation in dosages of 1Gy; 2Gy respectively. The effects of Olaparib treatment, the response to radiation, and the combined treatment of both were tested first on colony formation assays, than we performed "Annexin V dead cell assay kit" and "Cell cycle assay kit" of Merck to detect different forms of apoptosis.
Results: We detected that the viability of cell lines decreased significantly ($p<0.05$) after 8uM, 4uM and 2uM Olaparib treatments on MTT tests. Also, the pretreatment with Olaparib resulted more effective radiation on MTT tests. We observed a significant cell number reduction with colony formation assays. The presence of living cells decreased significantly and the early and late apoptotic forms of cells increased after Olaparib treatment significantly.
Discussion: Olaparib is an FDA- approved targeted therapy for ovarian cancer, accordingly to test its effect on other cell lines is necessary.
Conclusion: Olaparib pretreatment strengthened the antiproliferative effect of radiation on cervical adenocarcinoma cells *in vitro*. The molecular biological background of the above-mentioned results should be the subject of further examinations.

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Comparative pharmacokinetic study of levocetirizine in pregnant and non-pregnant rats

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Keywords: HPLC, pharmacokinetic study, pregnancy, levocetirizine

Introduction: Approximately 10-30% of the global population has allergic diseases. Pregnancy may alter the pharmacokinetics of drugs, which can affect fetus causing harm. Drug treatment needs a special concern in this condition. Levocetirizine is a third generation H1-antihistamine drug classified in FDA pregnancy risk category B. The aim of this study was to compare the pharmacokinetic properties of levocetirizine in pregnant and non-pregnant rats.
Methods: Levocetirizine was administered per os to 20-day pregnant and non-pregnant Sprague-Dawley rats at a dose of 10 mg/kg. Blood samples were collected from the tail vein at 10, 30 min and 1, 2, 4, 8 and 24 hours after treatment. Fetal blood samples also were taken at 1 and 2 hours after drug administration. Levocetirizine plasma concentrations were determined by high performance liquid chromatographic (HPLC) method on a KinetexC8 analytical column with 0.01 M Na_2HPO_4 buffer – methanol mobile phase mixture. The absorbance of levocetirizine and internal standard diazepam were detected by a photodiode array detector at a wavelength of 230 nm.
Results: Pharmacokinetic parameters were calculated from plasma levels using PKSolver 2.0 software. The levocetirizine concentration at the elimination phase of plasma concentration-time curve was significantly higher in the case of pregnant rats. The absorption rate constant (k_a), volume of distribution (V_D), the $AUC_{0\text{-}24h}$ value and the elimination half-life ($t_{½}$) of levocetirizine were increased, while elimination rate constant (k_e) and total body clearance (Cl_T) of the drug were decreased in pregnant rats as compared with non-pregnant animals. Levocetirizine crosses the placenta and enters fetal circulation.
Discussion: There is no significant difference between the absorption phases of the plasma concentration-time profiles of the two groups. The elimination of levocetirizine is significantly slower in pregnant rats compared to non-pregnant animals. This may either indicate a lower capacity of the pregnant rats to metabolize the drug or a slower efflux of levocetirizine from the placental and fetal compartment back into the maternal circulation.
Conclusion: In view of the slower elimination and increased bioavailability of levocetirizine in pregnant rats, further study is warranted to determine whether dose reduction is necessary in human pregnancy for safer therapy with the maintenance of efficacy.

Ethical Committee Approval: All experiments involving animal subjects were carried out with the approval of the Hungarian Ethical Committee for Animal Research (permission number: IV/198/2013).
Acknowledgement: The study was supported by Cedars Sinai Medical Center's International Research and Innovation in Medicine Program, the Association for Regional Cooperation in the Fields of Health, Science and Technology (RECOOP HST Association) and the participating Cedars – Sinai Medical Center - RECOOP Research Centers (CRRC).

Changes in the cervical resistance by COX-inhibitors and alpha-tocopherol in rat

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Key words: NSAID, tocopherol, cervix, rat

Introduction: Vitamin E and their analogues can differentially affect the physiological mechanisms. In our previous study we already demonstrated that the alpha-tocopherol can push the COX-1/COX-2 ratio towards COX-2, which leads to more powerful relaxation effect of NSAIDs in pregnant rat myometrium. Our aim was to investigate how alpha-tocopherol influences the effects of COX inhibitors on cervical resistance in rats *in vitro*.

Methods: The cervical resistance of 22-day-pregnant and non-pregnant Sprague-Dawley rats was measured in isolated organ bath *in vitro*. Alpha-tocopherol-succinate (10^{-7} M) was applied, while non-selective diclofenac (10^{-6} M), COX-2 selective rofecoxib (10^{-6} M) and selective COX-1 inhibitor SC-560 (10^{-6} M) were used as COX-inhibitors. The COX activities of the cervix samples were measured by enzyme-immunoassay.

Results: The cervical resistance of estrous rats did not alter by tocopherol in either COX-inhibitor. In case of pregnant rats, the alpha- tocopherol alone decreased significantly the cervical resistance. Furthermore, both diclofenac and rofecoxib reduced more potently the resistance than tocopherol. It was also found that the resistance of cervix was kept on dropping when COX-inhibitors were used after the pretreatment of tocopherol. The total COX activity was the highest in control pregnant samples, while in the presence of tocopherol it decreased significantly. This difference can be explained by the COX-2 activity and reduction effect of tocopherol.

Discussion and Conclusions: The cervix consists of about 90 % collagen and about 10 % smooth muscle. However, at the end of pregnancy, the collagen is degraded while the level of smooth muscle is not changed. Hence, our results suggest that the decrease in cervical resistance was caused by the COX-2 inhibitory effects of alpha tocopherol and COX-inhibitors in late pregnant cervical smooth muscle.

Ethical Committee or Institutional Animal Care and Use Committee Approval: All experiments involving animal subjects were carried out with the approval of the Hungarian Ethical Committee for Animal Research (permission number: IV/198/2013).

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

Improvement of virtual screening efficacy with a property-based scoring scheme for finding glucocorticoid receptor modulators

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Key words: glucocorticoid receptor, selective glucocorticoid receptor modulator, ligand-based, molecular descriptor, desirability function

Introduction: Glucocorticoids (GCs) play an important role in the therapy of inflammatory and auto-immune diseases. However, their chronic usage is firmly limited due to their severe side-effects. After ascertaining that the therapeutic and the side-effects are derived from different glucocorticoid receptor (GR)-dependent mechanisms, further attempts to detach these pathways by replacing the steroid core by non-steroidal scaffolds were established. In our studies virtual screening of extant databases was carried out to find new lead molecules to develop selective GR modulator compounds (SEGRMs). A scoring scheme based on desirability functions to increase the efficacy of the process was defined.

Methods: To build the scoring scheme, 1999 compounds of ChEMBL database tested on human GR by radioligand binding assay were examined. Compounds with steroid structure were rejected. For retrieving the 3D structures and evaluating molecular descriptors Schrödinger Suite was applied. Actives were defined as molecules with less than 1000 nM of K_i values. Then, the database was randomly divided to a *training set* of 821 active and 1145 inactive compounds and a *test set* of 550 active and 753 inactive compounds were set up. The medians of the descriptors of active and inactive compounds were compared by Mann-Whitney U test to ascertain if the differences were significant at the $p<0.01$ level. For building the scoring scheme only those descriptors were kept where significant differences could be detected. For statistical evaluations, building the scoring scheme and plotting, Python 2.7 was utilized.

Results: According to statistical evaluations, 96 descriptors were kept to define desirability scores. Besides statistical evaluations, visual inspections of the data were carried out. Box plots and histograms were prepared for the 96 descriptors with separation of the data of actives and inactives and for the desirability scores as well. With desirability scores it could be forecasted if a given compound was active on GR or not.

Discussion and Conclusion: A scoring scheme was built with which the activity of random molecules can be predicted on GR by molecular descriptors. Nevertheless, further optimization of the method is being developed.

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Study of Viral Kinetics and Histopathology of CVB3 infection in C57BL/6 male mice

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Keywords: *coxsackievirus, mouse models, immune response, pathogenesis, virus replication*

Introduction: C57BL/6 mouse strain is a commonly used animal model for studying immune competence. This inbred mouse strain is significant for distinct responses after infection. In our laboratory we have studied viral pathogenesis on genetically different mouse strains and different routes of infection. In this study our aim is to follow up pathogenesis of CVB3 infection in inbred C57BL/6 male mice.
Method: 50 (3 weeks old) C57BL/6 male mice were infected with CVB3-Nancy strain, dose $2x10^6$ $TCID_{50}$ by the intraperitoneal (i.p.) route. Samples were collected at days 0, 3, 5, 10 and 45 days post infection (p.i.). Blood, heart, pancreas, thymus, spleen and brain were snap frozen for titration of replicating virus, for viral RNA detection by PCR (RT and Nested), and fixed in formaldehyde for monitoring histopathological and immunohistochemical analysis.
Results: We did not notice statistically significant differences in weights between the infected and control groups. Viral RNA in heart was detected at days 3 and 5 post infection (p.i.) and in pancreas until 10 day p.i. Infected mice showed persistence of viral RNA in the spleen till day 45 p.i. Histopathological results showed incipient pancreatitis in 3/5 mice at day 3 p.i., acute pancreatitis in 4/5 at day 5 p.i.; and mild infiltration and chronic pancreatitis in 3/5 at day 10 p.i. In brain tissues mild pericapsular oedema was observed in 12/20 mice.
Discussion: These results demonstrate differences in histopathological changes and viral kinetics in the organs of CVB3-Nancy infected C57BL/6 inbred mice and Swiss albino outbred mice infected with the same virus and same route of our previous study.
Conclusion: We conclude that the C57BL/6 strain male mice are more resistant to infection. Based on these observations we suggest that susceptibility to the virus depends on the route of infection and the mouse strain.

Institutional Animal Care: This study was conducted with approval of the Ethical Committee of the Slovak Medical University and the State Veterinary and Food Control Authority of the Slovak Republic,: 1.10.2016 to 30.12.2020. Number: C.k. Ro 3248/16-221.
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Relationships among different adherences of type 2 diabetic patients

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Keywords: type 2 diabetes, medication adherence, lifestyle adherence, self-efficacy, health literacy

Introduction: Therapy for patients diagnosed with type 2 diabetes mellitus is dependent on specific conditions and requires a high level of adherence. The success of diabetic therapy relies heavily on the motivation, psychological resources (i.e.: self-efficacy, health literacy, health locus of control) and adherence of the patients. The aim of our research was to explore the attitudes of the type 2 diabetes patients to medication and lifestyle recommendations as well as to gain a deeper insight into the role of adherence-determining parameters in disease management.

Methods: The present study involved 113 insulin resistant inpatients including 38 men and 75 women. The mean age of the patients was 60.56 years (*SD* = 12.94, minimum: 20 years, maximum: 85 years) and they all had been diagnosed with type 2 diabetes for an average of 13 years (*SD* = 8.230). In the course of the study, the participants' degree of adherence was measured using Morisky's Medication Adherence Scale (MMAS-4) and a questionnaire on Diabetes Adherence originally conceptualized by the research team with the help of psychological and psychosocial mapping parameters.

Results and discussion: On the basis of our results, it seems a high level of medication adherence negatively correlates with lifestyle adherence. Among the socio-demographic factors the duration of diabetic conditions and age impact the most medication adherence the most. Multivariate regression analyses have shown that blood glucose adherence is mostly determined by the social-external health locus of control, diabetes self-efficacy and internal health locus of control, while dietary adherence is dictated by the patient's sense of self-efficacy and the duration of the illness. Additionally, the understanding and relevant use of diabetes treatment is clearly associated with dietary adherence and patients' high levels of self-efficacy, while health literacy is mostly determined by internal health locus of control.

Conclusion: The present study shows that adherence to medication, diet, glucose monitoring and physical exercise entails different levels of health improvement in diabetic patients, and adherence levels may depend on numerous factors, which together affect the physician-patient communication and the course of a diabetes therapy.

Ethical Committee Approval: 5/2016 – SZTE, University of Szeged, Szeged, Hungary
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Anticoagulant action of fibrinogen-specific proteases in vitro

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Keywords: defibrinogenation, protease, coagulation, platelet aggregation

Introduction: Reduction of coagulability of blood plasma fibrinogen is an important issue for preventing of intravascular thrombus formation. We supposed that the application of fibrinogen-specific proteases could be novel way of anticoagulant therapy that reduces fibrinogen ability to form intravascular thrombus without removing circulating fibrinogen from the bloodstream. In this work we studied several proteases of different origin as the potential anticoagulant agents.

Methods: Three proteases were purified from cultural solution of *Bacillus thuringiensis* var. *israelensis* IMV B-7465, the crude venoms of *Agkistrodon halys haly*s and *Echis multisquamatis* using several techniques including ion-exchange and size-exclusion chromatography. Turbidity study and platelet aggregation study were performed using spectrophotometer Optizen POP and aggregometer SOLAR-2110 respectively. Activated partial thromboplastin time (APTT) test was performed by standard method.

Results: Identification of hydrolytic products of fibrinogen were performed by SDS-PAGE under reducing conditions with further immunoprobing using the monoclonal 1-5A (anti-Aα509-610), II-5C (anti-Aα20-78) and MALDI-TOF analysis on Voyager-DE. We demonstrated that proteases from *A. halys haly*s venom, culture media of *B. thuringiensis* and *E. multisquamatis* venom hydrolyzed fibrinogen splitting of Aα414-610, Aα505-610 and Bβ1-42 forming desAα414-610, desAα505-610 and desBβ1-42 fibrinogens. After thrombin action polymerization lag-period for desAα414-610 and desAα505-610 fibrins was prolonged in 3 and 2.5 times respectively compared to control. Fibrinogen desBβ1-42 did not form polymerized fibrin under thrombin action. However, platelet aggregation rate was not much affected in comparison to control meanings (45 %) and was estimated as 25 %, 32 % and 40 % in the presence of desAα414-610, desAα505-610 and desBβ1-42 fibrinogen respectively. Overall effect of studied proteases on coagulability of human blood plasma was estimated by APTT test. Proteases from *A. halys haly*s venom and culture media of *B. thuringiensis* taken in the concentration of 0.01 mg/ml and incubated during 15 min with human blood plasma prolonged the coagulation time in APTT test in 1.5 and 1.2 times respectively. Protease from *E. multisquamatis* venom at same conditions completely diminished blood plasma coagulability in APTT test.

Discussion: Studied proteases hydrolyzed fibrinogen at different sites and impaired their polymerization thus reducing coagulability of blood plasma. Protease from the venom of *E. multisquamatis* was the most effective anticoagulant agent. However moderate anticoagulant effects of other two proteases accompanied by their anti-platelet actions seem to be more promising for *in vivo* use of these enzymes.

Conclusion: All studied enzymes are promising anticoagulant agents that must be studied more precisely in models *ex vivo* and *in vivo*. In particular we plan to study the possible anticoagulant effects of these proteases immobilized on columns for hemodialysis.

Ethical Committee Approval: The volunteers signed informed consent prior to blood sampling according to the Helsinki declaration. This study was approved by Ethics Committee of the Zabolotny Institute of Microbiology and Virology (12.11.2017, N3).

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RECOOP RESEARCH IN PROGRESS
Part II

Oral Presentations

Characterization of the blood coagulation system of morbidly obese patients

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Keywords: hemostasis, obesity, D-dimer, soluble fibrin

Introduction: Severe obesity is known as a complex metabolic disorder that is predisposed to atherosclerosis and atherothrombosis. The aim of the present work was the selection of basic parameters of the coagulation system that are essential for laboratory testing in blood plasma of morbidly obese patients.
Methods: 24 morbidly obese patients (BMI≥35) were selected for the study. Soluble fibrin (SF), fibrinogen and D-dimer were quantified using immunodiagnostic test-systems developed at the Palladin Institute of biochemistry. Laboratory samples were produced by DiaProph-med. Alternatively, concentration of fibrinogen was determined by modified spectrophotometric method using thrombin-like enzyme from *Agkistrodon halys halys* venom. Level of protein C was measured using specific chromogenic substrate S2366 (pyroGlu-Pro-Arg-pNA) and activating enzyme from *Agkistrodon halys halys* venom.
Results: Approximately 80 % of patients had substationally increased fibrinogen concentration (5.6±1.8 versus 3.0±0.5 mg/mL in controls). In most cases hyperfibrinogenemia is considered as ~~an~~ evidence of chronic low-grade inflammation and increased risk of intravascular clotting. Approximately 20 % of patients had the level of D-dimer increased trice or more compared to control levels (100±50 ng/mL for developed test). However, we did not find any significant decrease of the level of protein C, which is the main anticoagulant factor. Also, only 20 % of patients had an increased concentration of SF.
Discussion: Increased fibrinogen concentration in blood plasma of morbidly obese patients allowed us to expect the risk of hypercoagulability and recommend regular laboratory testing of the basic parameters of the blood coagulation system. However, according to analysis of individual patients, in two samples with normal range of D-dimer decreasedof protein C level and accumulation of SF were observed indicatingpathological thrombin generation. On the other hand, high level of D-dimer (more than 250 ng/ml) in most cases was accompanied by accumulation of SF. As far as SF is evidence of generat ion of thrombin and production of fibrin, while D-dimer is the result of fibrinolysis of stabilized fibrin, one can conclude that those patients had well-balanced haemostasis.
Conclusions. More than 30 % of patients with normal level of D-dimer (100-200 ng/ml) had high risk of thrombosis according to high levels of fibrinogen and SF. Two patients also additionally had decreased level of protein C that was assumed as the result of treatment of intravascular thrombus formation. Simultaneous quantification of SF, D-dimer and protein C is the only confident way to predict the risk of thrombotic complications in morbidly obese patients.
Source. This work was carried-out in the frame of the basic theme of the Palladin Institute of Biochemistry of NAS of Ukraine "Study of regulation mechanisms of blood coagulation and fibrinolysis interplay with vascular and platelet hemostasis".
Ethical approval. Patients signed informed consent prior to blood sampling according to the Helsinki declaration. This study was approved by the Ethics Committee of National O.O. Shalimov Institute of surgery and transplantology, (02.08.2015, N4).
Acknowledgement. We thank Cedars-Sinai Medical Center's International Research and Innovation in Medicine Program, the Association for Regional Cooperation in the Fields of Health, Science and Technology (RECOOP HST Association) for their support of our organization as participating Cedars – Sinai Medical Center - RECOOP Research Centers (CRRC).

Capillary electrophoresis as a molecular diagnostics discovery tool for common mechanisms of diseases

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Keywords: Electrophoresis, Capillary, Biomarkers, Diagnostic techniques and procedures, Molecular biology; Disease

Introduction: Capillary electrophoresis to mine the human plasma N-glycome has emerged as an efficient molecular diagnostics discovery tool for new biomarker candidates. In recent years, several investigations linked aberrant plasma N-glycosylation to numerous diseases. Changes in galactosylation and sialylation levels of IgG predicted obesity related diseases (gestational diabetes) during pregnancy. Maternal IgGs are actively transported to the fetus by the neonatal Fc receptor expressed in syncytiotrophoblasts in the placenta, providing the fetus and newborn with immune-protection. There are significant differences in the total glycosylation profile between fetal and maternal IgGs, suggesting a possible glycosylation-selective transport via the placenta that should be comprehensively investigated. Other markers, such as the N-glycosylation of acute phase proteins should also be measured.
Methods: Capillary electrophoresis was used with laser induced fluorescent detection for *N*-glycosylation profiling in conjunction with the Fast Glycan Sample Preparation and Analysis kit and the PA 800 Plus system (both from SCIEX, Brea, CA).
Results: Our preliminary study addressed comprehensive N-glycosylation identification of four important acute phase proteins: haptoglobin, transferrin, α-antitrypsin, and IgA in addition to IgG. Detailed N-glycosylation structure determinations of these protein standards were accomplished in this phase of the work along with the analysis of their FFPE versions.
Discussion: The macro- and microheterogeneity of the N-glycosylation of these important biomarker candidate proteins let us establish the baseline for future studies addressing the N-glycosylation differences of obese maternal and fetal tissue samples in the next step of this research endeavor.
Conclusions: Existing clinical laboratory tests measuring plasma levels of IgG and acute phase proteins do not provide diagnostic and prognostic information about the amount or structures of the attached sugars, which may be unique for obese pregnant women and their newborns. In the next phase of this work, placenta sample N-glycomes will be compared in maternal and newborn plasma samples.

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Body Mass Index and Markers for Chronic Inflammatory Response: High Sensitivity C-Reactive Protein and Procalcitonin in the Third Trimester of Pregnancy

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Keywords: pregnancy, body mass indeks, C-reactive protein, procalcitonin

Introduction: It is well accepted that adiposity in general is associated with significantly higher circulatory levels of different inflammatory markers. The aim of this study was to investigate whether such correlation between proinflammatory markers and body mass index (BMI) is present in pregnant women as well.
Methods: 30 pregnant women in the third trimester of pregnancy were included in the present study. They were categorized based on BMI into three groups: 6 patients with BMI <24.9kg/m2 (BMI I), 11 patients with BMI 25-29.9 kg/m2 (BMI II) and 13 patients with BMI>30 kg/m2 (BMI III). The inflammatory markers (high sensitivity C reactive protein (hsCRP) and procalcitonin (PCT)) were measured between 28 $^{1/7}$ and 32 $^{0/7}$ weeks of gestation. Perinatal outcome was analysed in all participants.
Results: There was no differences in age and smoking habits between all three groups of subjects. The authors found statistically significant differences in hsCRP mean values between groups: BMI I 4.637 mg/l, BMI II 5.720 mg/l; BMI III 10.910 mg/l (P= 0.002). However, there were no statistically significant differences in PCT mean values between groups: BMI I 0.0218 ng/ml; BMI II 0.0229 ng/ml; BMI III 0.0261 ng/ml (P= 0.456). Regarding perinatal outcome, there were no differences in gestational age at the time of delivery and incidence of Intrauterine growth restriction (IUGR) between these three groups. The cesarian section rate tended to increase with higher BMI in pregnant women.
Discussion and Conclusions: Maternal obesity in the third trimester of pregnancy is associated with an increase of hsCRP with increasing BMI Although normal pregnancy exhibits proinflammatory features, this does not seem to have additive or synergistic effects on the inflammation associated with adiposity. Further research is needed to determine the connection between obesity-induced inflammation and maternal and fetal health.

Acknowledgments. We are grateful to our colleague from Clinic of Gynecology and Obstetrics and Institute of Clinical Laboratory Diagnostics for providing our results.
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Ethical Committee Approval: Ethical Committee of University Hospital Center Osijek, registration number 25-1:227-4/2011

Anti-inflammatory effects of the omega-3 fatty acids

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Key words: n-3 PUFAs, alpha-linolenic acid, arachidonic acid, inflammation

Polyunsaturated fatty acids (PUFAs) are divided into two groups of n-3 (α-linolenic fatty acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA)) and n-6 (linoleic fatty acid (LA) and arachidonic fatty acid (AA)) acids. ALA and LA are two essential fatty acids. ALA is found in seeds (chia, flaxseed, hemp) nuts (e.g. walnuts), and many common vegetable oils and fish. ALA does not usually accumulate in cellular/tissue lipids/phospholipids even when ingested at relatively high dietary levels, because it undergoes beta oxidation in the mitochondria and only a limited amount is available for the very limited conversion of ALA to EPA and DHA. Nevertheless, in observational studies, higher ALA exposure was associated with a moderately lower risk of cardiovascular diseases. ALA, EPA, and DHA are especially important for good condition of the heart and blood vessels, as well as for the prevention of diabetes, in metabolic syndrome, and certain types of cancer.

n-3 PUFAs have anti-inflammatory, hypolipidemic and antithrombotic properties, may decrease the pro-inflammatory action of adiponectin and slow down the development of atherosclerosis. Moreover, they also have a beneficial effect on digestion, improve the immune system, and reduce occurrence of allergic diseases. AA replacement by EPA or DHA results in reduced/inhibited production of pro-inflammatory mediators such as prostaglandins, leukotrienes, and lipoxins.

The anti-inflammatory effects of n-3 PUFAs via reduced production of pro-inflammatory mediators include reduced/inhibited leukocyte chemotaxis, reduced adhesion molecule expression, and leukocyte-endothelial interactions. In addition, among the products of omega-3 fatty acid metabolism are the resolvins, maresins, and protectins which have an unreplaceable role in the reduction of inflammation.

Control of environmental risks factors, good management of the occurring diseases and balanced nutrition can significantly improve the metabolism and decrease risks for development of cardiometabolic diseases with low-grade inflammation in the background.

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Changes in genetic and environmental influences on arteriosclerotic traits: a longitudinal twin study

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Keywords: vascular stiffness, atherosclerosis, carotid arteries, twins, ultrasound

Introduction: The genetic background of measures of arterial stiffness, wave reflection (aortic pulse wave velocity, aPWV; aortic augmentation index, aAIx) and carotid intima-media thickness (IMT) has been extensively described in cross-sectional studies. We aimed at evaluating the impact of genetic and environmental factors on longitudinal changes in the variation of some arteriosclerotic measures over time.
Methods: 368 twins (214 monozygotic, MZ and 154 dizygotic, DZ pairs, mean age at wave 1 51.9±12.8 years) from Italy and Hungary underwent oscillometry and ultrasonographic carotid IMT measurement in 2009/10 (first wave) and 2014 (second wave). aAIx as a measure of the arterial wave reflection, aPWV as a measure of arterial stiffness, heart rate (HR), and mean arterial pressure (MAP) were also assessed. Bivariate Cholesky models were fitted to decompose the total variance at each wave and covariance between waves into additive genetic (A), shared environmental (C), and unique environmental (E) components.
Results: For each trait, at least moderate longitudinal stability was observed, with correlations between waves ranging from 0.23 (IMT) to 0.60 (aAIx). MZ cross-twin/cross-wave correlations were all significant and, in general, substantially higher than the corresponding DZ correlations, with the latter being significant in only one case (aAIx). A model including additive genetic and unshared environmental influences (i.e. AE) best explained the longitudinal data for all the traits. Under this model, genetic continuity was the main source of longitudinal stability, with genetic correlation estimates between 0.36 (IMT) and 0.70 (aAIx). Overlapping genetic factors explained from 55% (aAIx) to 88% (aPWV) of longitudinal covariance of the traits.
Discussion and Conclusion: We demonstrated that, for all considered cardiovascular traits, longitudinal changes develop mainly under the effect of genetic and unshared environmental influences. Moreover, the genetic contribution to the longitudinal stability is high for aPWV and MAP, while moderate for the other traits.
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Ethical Committee Approval: Semmelweis University TUKEB 29/2009, ETT, 58401/2012/EKU [828/PI/12], Instituto Superiore di Sanitá, 2009/2014.
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The mechanism of 5-aminoimidazole-4-carboxamide ribonucleoside-mediated antiproliferative and differentiative effects on acute myeloid leukemia cells

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Keywords: acute myeloid leukemia, AICAR, autophagy, metabolism

Introduction: 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR) and metformin have been widely used as agonists of AMP-activated kinase (AMPK). Our previous study demonstrated that AICAR inhibited proliferation and increased expression of differentiation markers in the monocytic U937 cell line in an AMPK-independent manner. The aim of the present study is to test for the role of autophagy and metabolic changes in AICAR-mediated effects and to assess the sensitivity of primary acute myeloid leukemia (AML) samples to AICAR.
Methods: HL60 and U937 cells were treated with AICAR, all-trans retinoic acid (ATRA) and metformin. The number of viable cells was determined by hemocytometer and expression of differentiation markers by flow cytometry. Cells transfected with mRFP-GFP-LC3B were analyzed by confocal microscopy. Gene knockdown was performed using siRNA transfections. The level of proteins of interest was analyzed by Western blot. Glucose, lactate and ammonia concentrations were measured using commercially available kits. Mononuclear cells from bone marrow of AML patients were obtained by density centrifugation and seeded in liquid medium containing 50 ng/mL IL-3, IL-6, SCF and FLT3-L. Viability was assessed by MTT assay.
Results: AICAR and other differentiation agents increased autophagy flux and the autophagy inhibitor 3-methyladenine inhibited differentiation. Downregulation of PI3KC3 and Atg7 had no effects on the AICAR and ATRA mediated increase of differentiation markers. AICAR had no significant effects on glucose consumption and lactate production, but increased production of ammonia. The lack of glutamine and presence of glutaminase inhibitor significantly inhibited AICAR-mediated effects. *In vitro* profiling of the sensitivity of primary AML samples revealed a significant decrease in viability after 96 h treatment with AICAR.
Discussion and Conclusion: These results show that although differentiation agents induce autophagy flux, their effects are not mediated by the key proteins of the classical autophagy pathway. Preliminary data suggest that AICAR mediated effects do not depend on glycolysis but depend on the presence of glutamine and the activity of glutaminase. Furthermore, AICAR exhibits pronounced anti-leukemic activity on primary AML samples *in vitro*.

Source of research support: This work has been supported by the Croatian Science Foundation under the project IP-2016-06-4581
Ethical Committee Approval: The study design was approved by the Ethical Commitee of University Hospital Centre Zagreb and the University of Zagreb School of Medicine (date: March 23, 2017; 380-59-10106-17-100/94).

Association of mtDNA copy number and telomere length with lumbar disc degeneration: a twin study

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Keywords: telomere, DNA, Mitochondrial, spine, Magnetic Resonance Imaging

Introduction: Degenerative disc disease (DDD), a common condition characterized by progressive loss of the cells of human nucleus pulposus and extracellular matrix has been related to telomere length (TL) and aging. Although there is a genetic predisposition to the development of DDD, the role of mitochondrial DNA copy number (mtDNAcn) and TL in this process has remained unclear.
Methods: 92 Hungarian twins, members of the Hungarian Twin Registry (34 monozygotic, MZ and 12 dizygotic, DZ pairs, mean age 48±15 years) underwent lumbar spine MRI (Siemens Magnetom Verio 1.5T) and phlebotomy. After DNA extraction from peripheral blood mononuclear cells, absolute telomere length (kilobase per diploid cell) and absolute mitochondrial DNA copy number (number of circular DNA per cell) were analysed by the qPCR standard curve method. The presence and number of bulging discs was recorded on T1 and T2 weighted sequences and compared with mtDNAcn and TL.
Results: In twins with presence of disc bulging (n=40), mtDNAcn was not significantly higher than in twins without disc bulging (n=20) (189±61 vs. 160±67, p=0.09). TL was shorter in twins with disc bulging (189±87 vs. 236±106, p=0.07), especially in MZ twins (p=0.04 for both parameters). In 5 monozygotic pairs discordant for the presence of disc bulging, mtDNAcn and TL were insignificantly less and shorter (mean ΔmtDNAcn difference -11.1, p=0.783 and mean ΔTL -14.1, p=0.860) in the affected monozygotic twin.
Discussion and Conclusion: Albeit our population was small, but it seems that the presence of lumbar disc bulging might be related to higher mtDNAcn and shorter TL. Further research should be pursued to examine common genetic factors in the background of DDD.

Financial support, grants: none.
Ethical Committee Approval: Semmelweis University TUKEB 189/2014, ETT, 3583/2015/EKU [25/2015].
Acknowledgement: We thank Cedars - Sinai Medical Center's International Research and Innovation in Medicine Program, the Association for Regional Cooperation in the Fields of Health, Science and Technology (RECOOP HST Association) for their support.

BOHDAN MALANIAK Young Scientist Award Competition

Protein biocorona formation at the nanoparticle surface and consequent changes in their properties during medical application

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Aim

The main goals of the proposal are to create an algorithm of human protein biocorona formation at the nanoparticle surface and analysis of consequent changes in their properties. Also to investigate the biocorona effect, formed by blood plasma and its main protein components, such as albumin, on the neuromodulating properties of carbon nanodots, quantum dots, nanocrystals and maghemite nanoparticles.

Background:

The project team has a strong background in nanoneurotechnology, including several papers concerning comparative analysis of the neurotoxic potential of synthesized, native, and physiological nanoparticles. The Polish and Ukrainian groups have two joint papers.
Recently, we performed a comparative assessment of the neurotoxicity of different types of nanoparticles using a similar methodological approach. The particle effects on key characteristics of excitatory and inhibitory (glutamate- and GABA-ergic, respectively) neurotransmission were analyzed using isolated brain nerve terminals (synaptosomes). The experimental data revealed different changes in the release of glutamate and GABA; the ambient level and uptake of these neurotransmitters, the plasma membrane potential and synaptic vesicle acidification in response to application of different types of nanoparticles to nerve terminals. Individual ambient concentrations of glutamate and GABA in the synaptic cleft are maintained due to neurotransmitter uptake by the plasma membrane neurotransmitter transporters in nerve terminals (Borisova and Borysov 2016; Pastukhov et al. 2016). We revealed neurotoxic effects for several nanoparticle types and the strength of the effects decreased from carbon nanodots synthesized from β−alanine (Borisova et al. 2015) and thiourea (Borisova et al. 2017), nanodiamonds (Pozdnyakova et al. 2016), maghemite nanoparticles γ-Fe_2O_3 (Borisova et al. 2014; Horák et al. 2017), NaYF4 nanocrystals doped with Eu^{3+} (Sojka et al. 2017) to volcanic ash particles (Pozdnyakova et al. 2017).

Coherence with RECOOP Research Strategy

The present research is concerned with the RECOOP Nanobiotechnology Research Platform. As it is said, “the modern pharmaceutical market requires novel drug-delivery systems rather than more novel drugs. The development of targeted drug delivery using conjugated nanoparticles brings more drug molecules to the diseased sites, at the same time reducing the negative side effects of a systemic drug exposure”. Our study aims to determine the effect of biocorona formation at the nanoparticle surface on drug delivery properties.

Scientific justification

Nanoparticles can absorb proteins and different biomolecules. We suggest that the biocorona is formed at the nanoparticle surface during medical application, including nanoparticle inhalation and intravenous injection, thereby significantly changing the properties. Inside human nanoparticles obtain a self-assembling dynamic coating named the biocorona. Formation of the biocorona can significantly change functional properties of both nanoparticles and biomolecules of the corona *per se*. Dynamic nanoparticle-biomolecule associates are temporal nanocomplexes. The biocorona plays a great role in nanoparticle-cellular interactions, in development of consequent biological effects, accumulation and degradation of nanoparticles. Considering the above, it is a crucial need to recognize the molecular mechanisms of biocorona formation (Saptarshi et al. 2013). We also consider that the investigation of nanoparticle biocorona formation using cell culture is not an adequate methodological approach because of the existence of "friendly" proteins in the cellular incubation media. Nanoparticles that aim at an organ or a tumor, are carried by the bloodstream after being injected or inhalated, and acquire a biocorona of different proteins, that can be "unfriendly" for their destination target. It is also important to study this effect in consideration of nanoparticle biosafety.

Expected outcomes:

Experimental data on biocorona formation and biocorona-related changes in neuromodulatory properties of nanoparticles will be of value and used for prediction of their behavior, distribution, accumulation, clearance and specific effects in humans during medical application.

The important question that arises from the point of view of medical application will be answered during project realization:" How does the nanoparticle biocorona affect the properties of definite types of nanoparticles and if it can be replaced by the different physiological molecules can specific types of nanoparticles become more biocompatible after coating with the biocorona?"

Our results will contribute to the understanding of the process of nano-bio interrelationships, absorbance of biomolecules by the nanoparticles, biocorona formation and degradation. We will determine types of nanoparticles that can form the biocorona. Our results will be a basis for development of predictive models for efficiency and biosafety of the medical application of different nanoparticles.

Correlation between placental vascularization indices, sFlt-1/PlGF ratio and blood coagulation factors in pregnancy hypertension.

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Key words: pre-eclampsia, vascularization indices, sFlt-1/PlGF ratio, coagulation factors,

Aim. The aim of the present study is to identify the correlation between placental vascularization indices, soluble fms-like tyrosine kinase-1 (sFlt-1)/placental growth factor (PlGF) ratio, platelet aggregation and hypercoagulability in pregnancies complicated with chronic hypertension (CHT) and pregestational diabetes mellitus (preDM).
Background. Recent studies showed that the building of the vascular network is mediated by PlGF and sFlt-1; and suggested that the sFlt-1/PlGF ratio predicts pre-eclampsia (PE) and its related complications from only 1 to 4 weeks earlier.
Previously the Ukrainian group found that determination of levels of protein C, soluble fibrin and functionally inactive forms of prothrombin and individually selected qualifying tests (fibrinogen, D-dimer levels; factor X and ATIII activity) may support the deeper understanding of the pathogenesis of PE especially in the case of pregnancy hypertension.
Previously the Hungarian group in cooperation with the Osijek group found that there is good correlation between placental vascularization indices and the sFlt-1/PlGF ratio. The number of patients examined and the number of pre-eclamptic cases did not allow us to establish a predictive value for PE in the case of vascularization indices. Thus, an increase in the number of patients would allow us to reach that goal.
Scientific justification. PE is the most serious hypertensive disorder during pregnancy. It occurs in 3-5% of pregnancies. Pregnancies complicated with CHT and preDM have increased risk for PE. The high-grade activation of the blood coagulation system that could be observed during pregnancy often leads to the risk of cardiovascular diseases especially in the case of pregnancy complicated by hypertension (such as CHT).
Planned outcome. Our opinion is that the present study will provide a positive predictive value of vascularization indices in PE and essential information on the pathophysiological background of PE through the exploration of the correlation between sFlt-1/PLGF ratio, 3-DPD vascularization indices, haemostatic factors and aggregometric measurements. These results may be useful for a deeper understanding of the background mechanisms, and the possibility of earlier detection of PE in risk groups such as CHT and preDM.

Ethical Committee Approval: Human Investigation Review Board, University of Szeged, Albert Szent-Györgyi Clinical Center, approval number is: 32/2014

BOHDAN MALANIAK Young Scientist Award
Progress Report

Project Interim Report II
Bohdan Malaniak CSMC-RECOOP Young Scientists Research Grant 2018
Oksana Matsyura, Danylo Halytskyi Lviv National Medical University, Lviv, Ukraine

Project title: Polymorphic variants of interleukin-13 R130Q and interleukin-4 T589C in children with and without cow's milk allergy
Project period: 12 months
Project started: January 2, 2018
Project will end: January 2, 2019
Measurable outcome: The natural history of allergic disease and its potential for prevention merit close examination because of the explosive worldwide increase in the prevalence and morbidity of atopic disorders. Accessible markers to predict the development of atopic diseases are highly desirable but as yet are a matter of debate. We are looking for correlations between a family history of atopy, environmental factors, genetic tests and association with food allergy risk in the Lviv population. By focusing on project outcomes, distributions of IL-4 T589C and IL-13 R130Q genotypes, high serum levels of total IgE, lactalbumin specific IgE and lactoglobulin specific IgE we will determine the markers of atopy risk. The results of this study will be implemented in clinical practice for the prevention of food allergy in children.

Participants
Applicant young scientist: Oksana Matsyura
Applicant CRRC organization: Department of Pediatrics №2 in Danylo Halytsky Lviv National, Lviv City Children's Clinical Hospital
Supervisor: Lesya Besh
Collaborating CRRC organization #1: Slovak Medical University in Bratislava, Slovakia
Collaborating young scientist: Dana Kosorinová
Supervisor of the young scientists collaborating CRRC organization: Katarína Volkovová
Collaborating CRRC organization #2: Department of Food Chemistry and Nutrition, Institute of Food Quality, Safety and Nutrition; Faculty of Food Science, Szent István University, Budapest, Hungary
Supervisor of the young scientists collaborating CRRC organization: Prof. Dr**.** Livia Simon Sarkadi, full professor
Collaborating young scientist: Dr Nándor Takács, assistant professor

Aim of research is to investigate whether IL-13 R130Q and IL-4 T589C polymorphisms are associated with a risk of developing allergic diseases in the Ukrainian population. The genotypic and allelic distribution of these polymorphic variants will be determined in cow's milk allergy (CMA) patients and normal healthy individuals.

Original project plan
I. Epidemiologic study
Step 1. Epidemiologic analysis of the incidence of food allergy in children in the Lviv region (Ukraine). A questionnaire survey for the prevalence of food intolerance and food allergy in the Lviv region will be conducted in 2400 children *(January 2018)*.
II. In vitro and in vivo studies
Step 2. Complex diagnostic procedures at the ~~in~~ Lviv City Children's Clinical Hospital of 80 patients who submitted questionnaire information about CMA (about 20 patients will be excluded after diagnostic procedures) and 60 healthy children. We will perform *skin testing* for *immediate-type sensitivity by skin-prick tests* ("Immunolog"; Ukraine and "Diater", Spain) for domestic, epidermal, food, *fungal* and pollen allergens, e*stimation* of *circulating*

total IgE *level, specific IgE* antibodies in the blood serum ("R-Biopharm AG"; Germany) and an oral challenge test with milk *(February 2018)*.

III. Diagnostic tests.
Step 3. Genetic tests (interleukin-13 R130Q and interleukin-4 T589C) in Slovac Medical University: 120 patients *(February 2018).*
Step 4. Detection of calcium, magnesium, *potassium, sodium and iron in blood serum at the* Department of Food Chemistry and Nutrition, Institute of Food Quality, Safety and Nutrition; Szent István University, Budapest, Hungary: 15 patients in dynamic in 6 months *(August 2018).*

IV. Therapy.
Step 5. Research protocol will include two investigated groups (1 – CMA, 2 – healthy).
In the CMA group (n=60) will be two subgroups: A subgroup (n=30) will receive oral immunotherapy, B subgroup (n=30) will eliminate milk in the diet.
Healthy children (n=60) will be the control group *(run-in: February 2018 –March 2018; treatment period – 6 months).*

V. Follow up.
Step 6. Diagnostic patients in dynamic, compare the outcomes of treatment and results of laboratory tests *(August 2018 – September 2018)*.

VI. Conclusions.
Step 7. Summarize results. Conclusions *(November 2018 – December 2018)*.

Timely performed research activities
1., Epidemiologic analysis of the incidence of food allergy in children in Lviv region (Ukraine). The questionnaires for the prevalence of food intolerance and food allergy in the Lviv region were conducted in 2432 children. The data show us that environmental factors and family history of atopy are associated with high allergy risk.

2., Complex diagnostic procedure at Lviv City Children's Clinical Hospital of 64 patients with CMA and 60 healthy children (16 were excluded). For this group of children we performed skin testing for immediate-type sensitivity by skin-prick tests ("Immunolog"; Ukraine and "Diater", Spain) for domestic, epidermal, food, fungal and pollen allergens, estimation of circulating total IgE level, specific IgE antibodies in the blood serum ("R-Biopharm AG"; Germany) and oral challenge test with milk. The results show us that the most common allergens are milk, eggs, and wheat. It is good for ~~to~~ food allergy~~ic~~ patients and families to introduce foods when they are safely tolerated, larger studies are needed to determine if ingestion of baked milk is solely a marker of transient milk allergy, or an effective treatment to induce tolerance.

Present status of the expenditure

A. **Costs of epidemiologic analysis.**

Epidemiologic analysis of the incidence of food allergy in children in Lviv region (Ukraine). A questionnaire survey for the prevalence of food intolerance and food allergy in Lviv region was conducted in 2432 children.

Sub-total epidemiologic costs: $ 113.

B. **Laboratory tests.**

Enzyme immunoassay (EIA) for the quantitative determination of specific IgE antibodies in human serum. Estimation of *circulating specific IgE* antibodies in the blood serum ("R-Biopharm AG"; Germany) in 80 children.

Sub-total laboratory costs: $ 1232 (already funded by RECOOP).

C. **Justification of the request for travel and accommodation support**

In Lviv City Children's Hospital we obtained blood samples and froze them (-20 °C). Afterwards all samples were transported to the institutions of our partners in dry ice.

Genetic tests by Slovak Medical University in Bratislava (120 blood samples are delivered to this institution) will show us the distributions of IL-4 T589C and IL-13 R130Q genotypes between CMA children and healthy individuals in the Lviv population. We hope to find correlations between genetic results and high serum levels of total IgE, lactalbumin specific IgE and lactoglobulin specific IgE in patients suspected to have milk protein allergy in children with CMA.

Bratislava (Slovakia).

I visited Bratislava on 09/03/2018.

Detection of calcium, magnesium, potassium, sodium and iron in blood serum at the Department of Food Chemistry and Nutrition, Institute of Food Quality, Safety and Nutrition; Szent István University, Budapest, Hungary was done in 15 patients in dynamic in 6 months (blood samples are delivered to this institution). The results will show us the nutritive status of two groups of children – with elimination diet and oral milk tolerance. It will help us to provide recommendations for children with CMA ~~cow's milk allergy~~.

Budapest (Hungary).

I spent one working day 09/03/2018 at the ~~in~~ Department of Food Chemistry and Nutrition, Institute of Food Quality, Safety and Nutrition; Faculty of Food Science, Szent István University.

Sub-total cost for travel support Lviv – Budapest – Bratislava – Lviv (08.03.2018 – 10.03.2018): $ 230 (already funded by **RECOOP).**

Adjustment of the Project Timetable:

III. Diagnostic tests.

Step 3. Genetic tests (interleukin-13 R130Q and interleukin-4 T589C) in Slovac Medical University: 120 patients *(blood serum was delivered in February 2018).*

Step 4. Detection of calcium, magnesium, *potassium, sodium and iron in blood serum at the* Department of Food Chemistry and Nutrition, Institute of Food Quality, Safety and Nutrition; Szent István University, Budapest, Hungary: 15 patients in dynamic in 6 months *(February and August 2018).*

IV. Therapy.

Step 5. Research protocol includes two investigated groups (1 – CMA, 2 – healthy).

In CMA group (n=60) are two subgroups:

- A subgroup (n=30) – oral immunotherapy,
- B subgroup (n=30) – elimination of milk in diet.

Healthy children (n=60) are the control group.

120 patients were enrolled in the study protocol (run-in period: *February 2018 –March 2018)* and now are going to the treatment period (for 6 months).

V. Follow up.

Step 6. Diagnostic patients in dynamic, compare the outcomes of treatment and results of laboratory tests *(August 2018 – September 2018)*.

VI. Conclusions.

Step 7. Summarize results. Conclusions *(November 2018 – December 2018)*.

Expected outcomes.

The prevalence of food allergy in the world varies from 6% to 8% in children. We will compare this number in children in different environmental conditions in the Lviv region (Ukraine). We are looking for correlations between a family history of atopy, environmental factors and association with food allergy risk. The distributions of IL-4 T589C genotypes and allele will be different between CMA children and the control group. The frequencies of the CC genotype and C allele will be higher in allergic children compared with healthy individuals in the Lviv population. We expect that the distribution of IL-13 R130Q genotypes and A allele will be significantly higher in the food allergy group compared with healthy individuals. We are focused on correlations between genetic results and high serum levels of total IgE, lactalbumin specific IgE and lactoglobulin specific IgE in patients suspected to have milk protein allergy in children with CMA.

Project Interim Report II
Bohdan Malaniak CSMC-RECOOP Young Scientists Research Grant 2017-2018
Andrii Lozynskyi, Danylo Halytskyi Lviv National Medical University, Lviv, Ukraine

Project title: Superparamagnetic iron oxide nanoparticles as bio-imaging agent and carrier of anticancer drugs
Project started: September 2017
Phase I of Project will end in September 2018
Measurable outcome: Improved solubility of compound **Les-3288** without significant loss of antitumor potential and conjugation with superparamagnetic nanoparticles. After completion of Phase I could start Phase II.

Participants:
Applicant young scientist: Andrii Lozynskyi, PhD;
Applicant CRRC organization#1: Danylo Halytskyi Lviv National Medical University;
Supervisors: Roman Lesyk, PhD, ScD; Lesya Kobylinska, PhD;
Collaborating CRRS organization#2: Department of Polymer Particles, Institute of Macromolecular Chemistry, The Czech Academy of Sciences;
Collaborating young scientist: Maksym Moskvin;
Supervisor of the young scientist collaborating CRRC organization: Daniel Horak, PhD;

There was no change in the participating research scientist as cooperation went well during the research period.

Aim of the project:
The main goal of the proposed project is the design, development and application of novel effective drug delivery systems and study of the biodistribution and biocompatibility of a novel anticancer drug conjugated to magnetic iron nanoparticles.

Present status of the study:
Step 1 – The synthesis of superparamagnetic iron oxide nanoparticles with biocompatible polymeric coating containing free carboxyl groups for capturing water-soluble drugs (D. Horak and Team)
a., Superparamagnetic nanoparticles (20 nm) coated with a polymeric shell containing free carboxyl groups were synthesized by the team of Horak D. Michal Babic (Institute of Macromolecular Chemistry). They have prepared the iron oxide particles with poly(*N,N*-dimethylacrylamide-*co*-acrylic acid) coating (maghemite, ɣ-Fe_2O_3). The particle contains 4,4 mg/ml Fe_2O_3 and 4,4 mg/ml modifier (91% dimethylacrylamide and 9% acrylic acid). Ca. 7 nm size of the dry maghemite core according to TEM, hydrodynamic diameter in water according to dynamic light scattering amounts to ca. 120 nm, active groups are carboxyls, polymeric shell consists of 91 wt.% of N,N-dimethylacrylamide and 9 wt.% of the acrylic acid.

Step 2 – Design of water-soluble derivative of Les-3288 is a novel 4-thiazolidinone derivative whose potential anticancer activity will be proved at the National Cancer Institute in USA. Resynthesis of Les-3288 substance (R. Lesyk, A. Lozynskyi)

a., The performance and evaluation of the ability to bind of the tested compound with nanoparticles at an initial stage was carried out using Les-3833 as an analog of Les-3288.
As a first step we changed the composition of the matrix, i.e., in tetradecanol : poly(ethylene glycol)-block-polycaprolactam ratio:

17/022 Les-3833+SNP = 5 : 1
17/023 Les-3833+NP = 1 : 3
Tetradecanol is sensitive to heating to 40 ºC. The copolymer is a biocompatible surfactant (stabilizer) and general compatibilizer of the system. It looks like higher tetradecanol content leads to better results.

b., The sample of the target compound Les-3288 was received by the Kobylinska Team and searching continued for optimal reaction parameters for the creation of conjugates.

c., The synthesis of the target compound (**Les-3288**) was performed by using two alternative methods (Method A and B). In the first case the synthesis was accomplished by one-pot methodology involving reaction of 3,5-diaryl-thiocarbamoyl-pyrazolines with chloroacetic acid and appropriate carbonile compound in the presence of sodium acetate in refluxing acetic acid (Method A). It is known that nature of the ylidene moiety in position 5 of the 4-thiazolidinone cycle has an essential influence on the antitumor activity. Based on these observations and considering the high isatin reactivity as carbonile compound, we utilized an appropriate carbonile compound in the reaction with 4-pyrazolyl-1,3-thiazol-2(5*H*)-one according to the standard Knoevenagel condensation procedure. Following the mentioned reactions, the non-condensed isatin conjugate with pyrazoline and 4-thiazolidinone has been synthesized (Method B). It should be noted that the experimental results revealed that Method A was more favorable than Method B for the synthesis of target compound 77 and 55% yield, respectively.

d., In continuation of our project we studied the interaction between target compound **Les-3288** and alkaline solutions in ethanol medium providing to formation of product with cardinally different solubility in comparison with the starting compound. In addition, the compound **Les-3288Na** in preliminary screening results on rat glioma C6 cell line was 2-fold more active than **Les-3288** at 2 and 5 μM concentrations.
Step 2 is planned to finish at the end of June 2018

Expenditures (USD):

Reagents for the synthesis of the target compounds (Catalog Sigma Aldrich):

Name	Pack Size	Price, USD
5-Bromoisatin	25G	99,24
p-Anisaldehyde	500G	125,69
Acetophenone	1KG	56,92
Thiosemicarbazide	100G	38,64
Isatin-5-sulfonic acid sodium salt dehydrate	5G	73,37
4′-Aminoacetophenone	25G	28,40
4′-Piperazinoacetophenone	5G	51,06
Total		473,48

Total 473,78 USD

Reagents for the studying of intracellular molecular targets (Chk1 and Chk2) of the compounds:

#	Manufacturer	Catalogue	Description	Unit	Unit Price (EUR)
1	Cell Signaling Technology, Inc.	2360S	Chk1 (2G1D5) Mouse mAb (SPECIES X-REACTIVITY: H, M, R, Mk) (APPLICATIONS: W) (VTSZ: 3822000099)	100 µl	311,1 EUR
2	Cell Signaling Technology, Inc.	2662S	Chk2 Antibody (SPECIES X-REACTIVITY: H, M, R, Mk) (APPLICATIONS: W IP) (VTSZ: 3822000099)	100 µl	311,1 EUR

Manufacturer	Catalogue	Description	Unit	Q-ty	Unit Price (EUR)
Santa Cruz	sc-200700	BML-277 (CAS 516480- 79-8)	10 mg	1	33 600 Ft (105 EUR)
Santa Cruz	sc-364463	CHIR-124 (CAS 405168- 58-3)	2 mg	1	31 040 Ft (97 EUR)
Santa Cruz	Delivery Cost		order	1	11 520 Ft (36 EUR)

Total 860.20 EUR

Matching contribution (USD):

All needed reagents are indicated in P.3 (473.78 USD + 860,20 EUR).

Outcomes.

The target **Les-3288** compound **r**esynthesized. By a chemical modification, a possible mechanism for improving solubility without significant loss of biological activity has been established. The preliminary screening of biological activity in the rat glioma C6 cell line was performed and revealed the necessity for further investigations of the antitumor potential of the tested compound.

Plan for the next steps:

The first year of the project will end September 2018. Conjugation **Les-3288** compound with improved solubility with superparamagnetic nanoparticles completed and *in vitro* tested.

Phase II.

Step 3 – the examination of the biocompatibility of created nanoparticles *in vitro* (Horak D.) and *in vivo* (Kobylinska L.)

Step 4 – the conjugation of 4-thiazolidinone derivative (compound ID3882) that is most promissing regarding cytotoxic action towards glioblastoma tumor cells (Lozynskyi A., Kobylinska L.) will be carried out

Step 5 – imaging of biodistribution, clearance and crossing of BBB of injected drug-containing nanoparticles (Lozynskyi A., Gajovic S., Vari S.)

Andrii Lozynskyi Lviv,
29.03.18young scientist

RECOOP RESEARCH IN PROGRESS

Interactive Poster Presentations

Part III

Evaluating scientific excellence in relation to received international grants

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Keywords: international collaboration, responsible research and innovations, impact measurement

Introduction: The inequality of research outputs across the word is addressed by various measures, the main one being internationally awarded research or infrastructural grants. In Central and East Europe, the main international financial resources are provided through the European Union to the EU 13 members, non-EU H2020 participants and near-neighbors. There is currently no information on the impact of the international and national projects in biomedical research conducted in the Republic of Croatia. Existing models of top-level scientific institutions for impact assessment are difficult to apply to the Croatian research environment. This work aims to adapt those models to assess scientific excellence as an impact indicator of research projects awarded to the Croatian scientific community.
Materials and methods: The study analyzes research groups in Croatia, where those groups, which successfully attracted international grants are still not numerous. Subsequently, the group leaders would be divided into those receiving international financial support and those relying on national grants. The excellence score would be designed in order to compare the outputs and abilities of receivers vs. non-receivers of international grants.
Results: The qualitative approach to narratives of non-receivers indicated that they do not connect their reliance on national grants to any lack of scientific excellence. There is an important claim on a specific form of scientific excellence thriving on restricted resources, stating that the lack of resources promotes the intelligent design of the experiments (1,2). Subsequently, the creation of an excellence score, which could be easily adapted to the specific research environment, represents the major challenge of the study.
Discussion and Conclusion: The excellence score should include the research output, elements of knowledge transfer and educational benefits in biomedical research projects (3). The study is expected to contribute to the informed decisions on research strategies in Central and East Europe.

Literature:
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Activities of Research and Technology Transfer Office of the University of Zagreb School of Medicine

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Keywords: technology transfer, research support, translational research

Research and Technology Transfer Office (RTTO) functions within the Center for Translational and Clinical Research, established in 2009 by University of Zagreb School of Medicine (UZSM) and University Hospital Center Zagreb (UHCZ). It represents an important asset for translational research.

Institutional support for research is growing and evolving in Central and East Europe in the last decade, with higher demands for applications to competitive international calls for grants as well as with demands for development of technology transfer activities within the Universities and other public research organizations. In addition to the level of the entire University of Zagreb with a central TTO and complementary to it, the School of Medicine established its own Office, due to specificity of biomedical research, transfer of biotech inventions, size of the School and a need to work closely with scientists.

Main challenges for RTTO are a non-entrepreneurial background, working with clinicians over-loaded with their clinical duties, a "traditional" administration and overall need for culture-change. Together with a "raising awareness" phase, RTTO was dedicated to the strengthening of participation in international projects, introducing procedures and training of staff and researchers.

With the success of obtaining several large EU projects funded by the framework programs (FP) of the European Commission, UZSM strengthened its capacities in research support by employing new people, acquiring experience, skills and knowledge, introducing an IP management committee and IPR by-law. In addition, several Proof of Concept projects were funded as well as two invention-development grants.

UZSM was the FP7 coordinator of the OSTEOGROW project, and as a continuation coordinates H2020 project OSTEOPROSPINE, within which, a clinical trial with a proprietary drug is performed. This showed that we do have capable individuals, "champions", able to compete in the world scientific arena, who can also lead and inspire others.

New project Alliance4Life, H2020 "Coordinating and Support Action", gathered ten research institutions from nine EU13 countries. The project aims to stimulate institutional and national advances, in order to strengthen scientific excellence, improve management and technology transfer, retain national and attract international talents, and exchange good practices. The project is expected to contribute to the participant's improvement, but also to influence and support other institutions in Croatia and in other transition countries.

Retracted papers of the Croatian authors in the international bibliographic databases

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Keywords: research integrity, publication ethics, paper retraction

Introduction: There has been an increase in the number of retracted papers in the international bibliographic databases. We aimed to identify the retracted articles of Croatian authors published in PubMed and Web of Science Core Collection (WoS CC) covered journals.
Materials and methods: PubMed was searched by the combination of ”Croatia“ in the address field and ”retracted publication“ or ”retraction of publication“ in the publication types field. The search of WoS CC was a combination of the address and title words – ”retract“ or ”withdraw“ and also – ”article“ or ”paper“ or ”publication“.
Results: In the WoS CC database we found 13, and in PubMed 11 retracted papers, 6 of them overlapping in both databases. The analysed sample consisted of 13 retracted papers, 4 of them published by Croatian journals, and 9 by international ones. Seven papers belong to the field of human medicine, 2 to the field of biology, and 1 to each of veterinary medicine, physics, chemistry, and education fields.
Discussion and Conclusion: Bearing in mind that the number of papers retracted by PubMed indexed journals has been near 1000 papers/year in the last 5 years, the numerical value of the Croatian retracted papers is relatively small. In addition, Croatian authors were publishing around 1500 papers/year in the PubMed indexed journals and the number of retracted papers is proportionally small.

Ethical approval: Not needed.

Relation between the antiquity of intracoronary thrombi and prognosis of patients with acute ST segment elevation myocardial infarction

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Keywords: percutaneous coronary interventions, manual thromboaspiration, intracoronary thrombi.

Introduction: Primary percutaneous coronary interventions (PCI) are the main method for the restoration of blood flow in patients with acute ST-segment elevation myocardial infarction (STEMI). But an optimal clinical result cannot be obtained by even PCI sometimes. A different duration of the thrombotic process may be the explanation for this.
Methods: The study included 20 patients with STEMI (3 women / 15 % and 17 men / 85 %) aged 53.90 ± 1.42 years old, who had been undergone PCI with manual thromboaspiration within the first 12 hours (mean 5.85 ± 0.69 h) after symptom onset. The obtained intracoronary thrombi (IT) were examined morphologically after hematoxylin & eosin staining by the standard method and also by the method proposed by prof. Zerbino. The latter one allows determining the age of fibrin, i.e., fibrin that had been formed within the last 24 hours ("fresh") is stained in red or violet and more than 24 hours ("old") is stained in blue. The IT were recognized as "old" if a part of fibrin had been stained in blue. Short term prognosis was defined by the frequency of ST-segment resolution, blood flow TIMI 3 grade after procedure and ejection fraction of the left ventricle (EF).
Results: The "old" IT were revealed in 14 (70%) patients. ST-segment resolution was found in 7 (50%) of these patients. ST-segment resolution was detected in all patients with the "fresh" IT. The difference between groups were statistically significant (p=0.032). Blood flow TIMI 3 grade was achieved in 9 (64.29%) patients with the "old" IT and in all with the "fresh" ones (p=0.091). EF during hospitalization wasn't different between the patients with the "old" vs. "fresh" IT (47.69%±2.63% vs. 51.67%±3.11%, respectively; p=0.467). EF slightly improved in the patients with the "fresh" IT in a year (52.33%±3.58%; p=0.371). It decreased in the patients with the "old" ones at the same time (45.75%; p=0.182). But the difference remained not significant (p=0.125). 2 (10%) patients died during the follow-up. Both of them had "old" IT (p=0.33).
Discussion. The age of IT is the best index to evaluate the duration of the thrombotic process. Even in patients with chest pain duration less than 12 hours sometimes we confirmed duration of the clot formation of more than 24 hours. Often the IT formation starts a day or more before the symptoms onset.
Conclusions: The "old" IT were often revealed during PCI in patients with STEMI duration less than 12 hours. They were associated with worse ST-segment resolution and have a trend toward worse indexes of EF.
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The influence of atorvastatin on omentin level and HOMA-IR in patients with coronary artery disease and obesity

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Key words: obesity, atorvastatin, insulinresistance, omentin-1.

Introduction: Obesity has reached epidemic proportions worldwide and is associated with numerous comorbidities, including coronary artery disease (CAD).
CAD is a major cause of death and disability in developed countries and therefore, requires timely treatment and prevention. Statin therapy significantly reduces the risk of cardiovascular events but may induce impaired glucose tolerance and insulin resistance. Omentin-1 is an adipokine which could have a protective role against the manifestation of atherosclerosis and may modulate insulin action.
The aim of the present study was to investigate the effect of atorvastatin on serum levels of omentin-1 and HOMA-IR in patients with CAD.
Material and Methods: The study included 20 patients (15 men and 5 women) with CAD and obesity aged 46-72 years. In all subjects glucose, insulin, HOMA-IR, HbA1c, lipids and omentin-1 were measured before the study and after 12 months. We tested omentin-1 in human serum by the ELISA method. Patients were treated with atorvastatin (20-40 mg/d) during 12 months.
Results: After 12 months we observed a significant increase in insulin resistance (3,01±0,47and 3,6±0,51; p=0,035) and impaired fasting glucose (6,1±0,21 and 6,64±0,27mmol/l; p=0,02) and elevated omentin-1 (0,4±0,08and 1,76±0,45 ng/ml; p=0,01). The lipid levels, and HbA1c were nearly identical.
Discussion: Our studies support the hypothesis that statins may cause altered glucose homeostasis. Also, we observed increased serum omentin-1 concentrations in our patients after 12 months.
Conclusions: The increment of serum omentin-1 levels with atorvastatin administration in patients with CAD and obesity has a protective role against atherosclerosis. For an understanding of the multiple roles played by omentin more studies are required.

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Ethical Committee or Institutional Animal Care and Use Committee
Approval:18/01/2016 № 1

Antiviral activity of sophorolipids against MHV-68

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Keywords: sophorolipid, herpesvirus, Murine gammaherpesvirus-68, antiviral

Introduction: Gammaherpesviruses are lymphotropic viruses associated with development of lymphoproliferative diseases. Currently, there is no available effective antiviral agent against viruses of the subfamily *Gammaherpesvirinae*. Sophorolipids are surface active glycolipids produced by non-pathogenic yeasts. They consist of a hydrophobic sophorose connected with a hydrophilic fatty acid by a β-glycosidic bond. Sophorolipids have antibacterial, antifungal, immunomodulatory and some antiviral activity. The aim of our study was to identify the antiviral activity of the sophorolipid g-citronellal and the curcumin-sophorolipid (CSL) against murine gammaherpesvirus 68 (MHV-68), which is genetically and biologically related to human gammaherpesviruses.
Methods: To investigate the biological activity of g-citronellal and CSL, the cytotoxic activity was determined by MTT assay. We established the highest non-toxic concentrations of substances in the Vero cell line. As an initial screening of antiviral activity the cytopathic effect reduction assay was done in Vero cells. We performed the plaque reduction assay to quantify antiviral activity of sophorolipids.
Results: Sophorolipid g-citronellal and CSL show a slight degree of antiviral activity against MHV-68. The presence of both substances induced phenotypic changes of MHV plaques: the plaques were significantly smaller and not sharp, compared to control virus plaques.
Discussion: MHV-68 is widely used as a model to study replication and transformation of human herpesviruses (Epstein-Barr virus, human herpesvirus associated with Kaposi sarcoma). If the antiviral activitiesof the tested sophorolipids against MHV-68 are confirmed, these sophorolipids can be used as effective antiviral agents against some human herpesviruses. G-citronellal and CSL induced a change in size and shape of the plaques, but not in their quantity. The compounds did not prevent plaque formation, therefore we suggest a slowing down influence on the replication process of MHV-68. A comparison with RNA virus and deeper study of the replication process are required.
Conclusion: We suggest that g-citronellal and CSL limit MHV-68 replication.

Ethical Committee Approval: was not required.

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Genetic versus environmental effects on the relationship between lumbar degeneration and low back pain: results of an MR twin study

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Keywords: spine, Magnetic Resonance Imaging, back pain, heritability, genetics

Introduction: 90% of low back pain (LBP) is referred to as non-specific, caused by degenerative spine disease without any exact pathology and triggering factors. Our aim was to study the genetic and environmental correlation between LBP and signs of lumbar degeneration.
Methods: 210 members of the Hungarian Twin Registry (63 monozygotic, 42 dizygotic, 135 female, 75 male, mean age: 52±14 years) underwent lumbar spine magnetic resonance imaging (MRI) (Miskolc: Siemens Magnetom Verio 1,5T, Budapest: Philips Ingenia 1,5T) and completed a validated questionnarie on LBP. T1 and T2 weighted images were evaluated using the MicroDicom free DICOM viewer. The scores of each degenerative lesion were summed up separately for the entire lumbar region. SOLAR v. 7.6.4 was used to estimate the heritability adjusted for age and sex.
Results: 91.6% of the population had experienced LBP ever in their life, and 73.2% had at least one disc bulging. The Pfirrmann score showed the strongest additive genetic influence ($h2=0.73$, $p<0.001$), while the ever-present back pain and its number was moderately heritable ($h2=0.33$, $p<0.05$). The total endplate score (TEPS) and the number of disc bulgings were mostly defined by common environmental factors ($c2=0.52$, $p<0.05$; $c2=0.47$, $p<0.1$). There was a significant environmental correlation between Pfirrmann score and the number of disc bulgings ($pE=0.32$, $p<0.05$). The genetic correlation between ever-present back pain and TEPS or Pfirrmann score was higher than the environmental ($pG=0.68$, p=ns), as well as between Pfirrmann score and TEPS ($pG=0.46$, p=ns). The back pain showed correlation with Pfirrmann score only ($pG= 0.4$, p=ns).
Discussion and Conclusion: The Pfirrmann score, marker of disc degeneration, was mostly defined by genetic factors, whereas the number of disc bulgings was mostly defined by common environment. Ever-present back pain is most commonly associated with TEPS or Pfirrmann score as MRI findings. Our results may help us understand why we see disc degeneration in asymptomatic patients so often, whereas various degenerative disorders could be prevented with ~~a~~ proper lifestyle interventions with various efficacies.

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Association between obstructive sleep apnea and lumbar disc degeneration: evidence of a genetic link

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Keywords: sleep apnea, spine, Magnetic Resonance Imaging, back pain, heritability

Introduction: Obstructive sleep apnea (OSA) is one of the major causes of excessive daytime sleepiness and cognitive dysfunction, and it increases cardiovascular morbidity and mortality. Low back pain (LBP) is often inflicted by lumbar degeneration and the prevailing chronic pain causes poor sleep quality and difficulties in falling asleep. Previous studies suggested a link between poor sleep quality and LBP, but no objective genetic study was conducted to understand the common pathway.
Methods: 71 Hungarian twin pairs from the Hungarian Twin Registry (42 monozygotic, MZ and 29 dizygotic, DZ pairs, mean age 51±16 years) underwent lumbar spine MRI (Siemens Magnetom Veria and Philips Ingenia 1.5T) and overnight polysomnography (Somnoscreen Plus Tele PSG, Somnomedics GMBH, Germany). Apnea hypopnea index (AHI), respiratory disturbance index (RDI) and oxygen desaturation index (ODI) were registered. Daytime sleepiness was measured by Epworth Sleepiness Scale and LBP was assessed by a validated questionnaire. The presence and number of bulging discs and total endplate score (TEPS) were recorded on T1 and T2 weighted MRI sequences and summarized. Univariate ACE modelling was applied.
Results: Prevalence of OSA and disc bulging were 39% and 68%, respectively, in our study population. Parameters of sleep quality and sleepiness showed a substantial additive genetic background (AHI, ODI, RDI between 39% and 94%, $p<0.05$, Epworth scale: 27%, $p<0.2$). The correlation between the number of bulging discs and sleep parameters showed a significant positive genetic correlation (REM AHI and TEPS: 0.59; REM AHI and disc bulging: 0.69; AHI and back pain: 0.64).
Discussion and Conclusion: OSA parameters are heritable. The presence and severity of OSA are related to the number of disc protrusions which has a common genetic background. Further research is needed to examine common genetic factors in the background of this relationship.

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Size of the pituitary gland and its connection with body mass index: genetics or environment?

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Keywords: twins, heritability, pituitary gland, body mass index, magnetic resonance imaging

Introduction: Several studies have analyzed the pituitary volume by magnetic resonance imaging (MRI) to determine standards for age, gender, but its heritability has not been investigated yet. Literature data revealed a strong link between primary empty sella and obesity, but no genetic study has been performed to understand the pathomechanism.
Methods: 118 healthy members of the Hungarian Twin Registry (36 pair monozygotic, MZ, 24 pair dizygotic, DZ twins, 74 women, mean age 50±14 years) underwent an unenhanced sella MRI (Philips Ingenia 1,5T). The diameters of the pituitary gland were measured manually, and its volume was calculated by the following formula: 1/6pi*height*length*width. Heritability was analyzed by variance component modelling with SOLAR v.7.6.4., adjusted for age and gender (Model-1) and also for BMI (Model-2).
Results: The mean value of pituitary volume was 502±201 mm^3. Partial empty sella was present in 12 subjects (10.3%). No additive genetic component was found in the anteroposterior diameter (Model-1: c^2=0.42, Model-2: c^2=0.43), but genetic factors were responsible for most of the variance of other diameters (h^2 between 0.48 and 0.67, $p<0.05$) and for the volume (Model-1: h^2=0.57, $p<0.001$, Model-2: h^2=0.59, $p<0.001$). In Model-2, adjusted for BMI, results did not show substantive changes. A bivariate analysis between the hypophyseal parameters and BMI revealed that the genetic correlation was higher than the environmental (pG between -0.23 and -1, pE between -0.1 and -0.25, p=ns).
Discussion and conclusion: Prevalence of partial empty sella is relatively common in our population. Genetic factors play a considerable role in most of the diameters and the volume of the pituitary gland, and the variance is not influenced by BMI. However, between the hypophyseal parameters and BMI a negative genetic correlation is present, indicating that patients with higher BMI tend to have a smaller pituitary gland, and genetic factors may have an important role in its background.

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Research and development of gingival gels on the base of ornidazole, chlorhexidine bigluconate, xylitol and essential oils

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Keywords: ornidazol, gel, periodontal diseases

Introduction: There has been a tendency for steady growth of various pathologies of periodontal tissues in recent decades. Microorganisms of the dental plaque are one of the reasons of inflammatory periodontal diseases. Therefore, the elaboration of antimicrobial gingival gels without preservatives is a topical issue of modern dentistry.
Methods: Biopharmaceutical microbiological methods for the study of releasing chlorhexidine bigluconate from the gels, potentiometric method for adjusting the pH of the gels, observation method at applying.
Results: Technological and microbiological biopharmaceutical research studies of the gingival gels were performed and justified. These studies demonstrated a significant effect of the carbopol concentration on the release of active substances, primarily chlorhexidine bigluconate. As the carbopol concentration increases from 1 to 2 %, antimicrobial activity reduces to the following pharmacopoeia test strains of the microorganisms: *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Candida albicans,* while such microorganisms as *Escherichia coli* and *Bacillus subtilis* were sensitive to the developed gels in depending on carbopol 980 concentration. A preliminary clinical study of the proposed extemporaneous gingival gels in a small cohort of patients (20) showed the effectiveness of the application in the initial therapeutic phase according to PMA, plaque and bleeding indices. The written informed consent had been obtained from the patients.
Discussion: Ornidazole, chlorhexidine bigluconate, xylitol and essential oils seem to be an attractive combination for treatment of oral cavity diseases, caused by periodontal anaerobic bacteria. However, it is necessary to perform wider nonclinical and clinical studies to compare sensitivity of the developed gels with authorized ones on the basis of metronidazole and chlorhexidine bigluconate. One of the advantages of the developed gels composition is an absence of preservatives.
Conclusion: The present study revealed prospects for planning local manufacture of the developed gels for treatment of oral cavity diseases, caused by periodontal anaerobic bacteria.
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Ethical Committee or Institutional Animal Care and Use Committee Approval: 24/01/2011 № 1 DHLNMU.

The role of component resolved diagnostics in treatment and prevention of food anaphylaxis in pediatric practice

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Key words: food anaphylaxis, cofactor, component resolved diagnostics, adolescent.

Introduction: Anaphylaxis is a severe life-threatening form of a generalized or systemic reaction of hypersensitivity. Anaphylaxis has been seen to develop in 0,5 - 2,0% of people. Food is one of the main triggers in the development of anaphylaxis. According to current data cofactors play a fundamental role in about 30% of the anaphylactic reactions.

Methods: A detailed study of 12 history cases with food anaphylaxis was done. We performed an estimation of circulating IgE antibodies in the blood serum for domestic, epidermal, food, fungal and pollen allergens ("R-Biopharm AG"; Germany) and investigation of component resolved diagnostics to related allergen components (ImmunoCAP specific IgE blood test, Pharmacia Diagnostics AB, Sweden).

Results: A thorough collection of the history of the disease, as well as a multi-component diagnostic approach allowed the detection of a high level of sensitization to Tri a 14 (wheat flour, omega-5-gliadin), Art v3 (wormwood), Ara h 1, Ara h Ara h 9 (peanuts) or Gly m5, Gly m 6 (soy) in 83,33% of patients. All these allergens belong to the class of storage protein or lipid transporting proteins (LTP), which are stable under heat treatment and the effects of hydrochloric acid and are associated with severe and systemic reactions.

Discussion: Frequent causes of allergic reaction initiation are physical activity, alcohol and medicines. On the basis of a comprehensive survey, all patients have to receive clear recommendations for the specificity of diet, lifestyle and regarding one's health risks.

Conclusions:
1. Component resolved diagnostics increases our possibility to determine cause and prevent food allergic reactions in patients with a more individual approach.
2. Cofactors are important in the development of anaphylactic reactions.
3. Patients with a risk of anaphylaxis development should be provided with the automatic syringe pens containing adrenaline and be informed by the physician about the method of their application.

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The ways for improving the bioavailability among biologically active 4-thiazolidinones and their structure-related analogs

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Key words: 4-thiazolidinones, cytotoxicity, anticancer activity.

Introduction. The synthetic approaches and biological activity evaluation of heterocyclic compounds containing 4-thiazolidinone fragment and their structure-related analogs are based upon previous systematic results especially in elaboration of potential chemotherapeutic agents. Thus, anticancer activity of 4-thiazolidinones can be associated with their affinity to JSP-1 phosphatase, TNFα, anti-apoptotic biocomplex Bcl-XL-BH_3, integrin $\alpha_v\beta_3$ receptor, PPAR-γ receptor, SHP-2 tyrosine phosphatase etc. Nevertheless, the high level of pharmacological potency is associated with slight bioavailability of these derivatives which complicated the usage of this type of compounds in modern chemotherapy.
Methods. The synthesis has been performed and the synthetic procedure of obtaining salts with better bioavailability of biologically active 4-thiazolidinone derivatives was adopted. The synthesized compounds and their salts were evaluated for anticancer activity on rat glioma C6 cell lines (Les-3288, Les-5637, Les-5884). In addition, the salt of compound Les-1351 was covalently attached to the lectin of pea seeds (*Pisum sativum* agglutinin) and screened on HEK-293, MCF-7 and L1210 cancer cell lines.
Results. The target pyrazoline-thiazolidinone (Les-3288), thiazolo[4,5-*b*]pyridine (Les-5637, Les-5884) and thiopyrano[2,3-*d*]thiazole derivatives (Les-1351) were synthesized via reactions [2+3], [3+3], [4+2]-cyclocondensations as well as Knoevenagel condensation. The synthesized compounds were transformed into corresponding salts in the reaction with alkaline solutions in ethanol medium. The preliminary anticancer screening results revealed that the relative number of living cancer cells has been decreased in experiments with treating salts of biologically active 4-thiazolidinones as compared to starting compounds.
Discussion. The tested 4-thiazolidinone derivatives and their structure-related analogs demonstrated promising activity in the *in vitro* screen on the tested cell lines, as well as some distinctive patterns of selectivity. In addition, empirical structure-activity relationship revealed that the presence of pharmacophoric groups in combination with fragments which improve bioavailability is favorable for cytotoxic potency against C6, HEK-293, MCF-7 and L1210 cancer cell lines.
Conclusion. The tested compounds displayed prominent cytotoxicity effects on chosen cancer cell lines, which revealed the necessity for further investigations among these derivatives in the modern drug discovery process.

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Beekeeping products as active substances of medicinal products

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Key words: beebread, propolis, flavonoids, antimicrobial activity

Introduction. Beebread and propolis have been used in traditional medicine and supplementary nutrition. Flavonoids of beebread and propolis are the largest group of phenolic compounds with antioxidant, antimicrobial, antiviral, antitumor, and anti-inflammatory activities. Therefore, determination of flavonoid content, and antimicrobial and antiviral activity is prerequisite for development of medicinal products for treatment of oral cavity infectious diseases. The primary aim of this study was to measure the flavonoid content of 16 beebread extracts and determine the antimicrobial activity of these extracts as well as to assess the literature data regarding propolis antiviral activity.
Methods. Total flavonoid content and antimicrobial activity of beebread extracts were determined by methods of differential spectrometry and disc diffusion, respectively.
Results. The flavonoid content of the extracts ranged from 8 to 198 mg in 1 L with the reference to quercetin. Fifteen strains of microorganisms were tested in this study: 3 Gram-negative bacteria (*Haemophilus influenzea* CCM 4456, *Klebsiella pneumoniae* CCM 2318, *Salmonella enterica* subs. *enterica* CCM 3807), 3 Gram-positive bacteria (*Bacillus cereus* CCM 2010, *Clostridium perfringens* CCM 4435, *Staphylococcus aureus* subs. a*ureus* CCM 4223), 3 yeasts (*Candida albicans* CCM 8186, *Candida glabrata* CCM 8270, *Candida tropicalis* CCM 8223) and 6 microscopic filamentous fungi (*Aspergillus clavatus, A. flavus, A. versicolor, Penicillium expansum, P. chrisogenum* and *P. gruseufulvum*). Microbiological studies showed that the Gram-positive bacteria were the most sensitive to the tested extracts. With the purpose of studying propolis antiviral activity, its extracts are prepared and investigated.
Discussion. Beebread and propolis seem an attractive source of antioxidants and components with antimicrobial action. However, it is necessary to perform wider studies to control sensitivity of more dangerous microorganisms and establish correlation between flavonoid contents and antimicrobial activity.
Conclusion. The present study revealed prospects for the development of dosage forms of beebread and propolis for treatment of oral cavity diseases.

Acknowledgement: We thank Cedars-Sinai Medical Center's International Research and Innovation in Medicine Program, the Association for Regional Cooperation in the Fields of Health, Science and Technology (RECOOP HST Association) for their support of our organization as participating Cedars-Sinai Medical Center-RECOOP Research Centers (CRRC). Nataliia Hudz thanks the International Visegrad Fund for the realization of research.
Ethical Committee or Institutional Animal Care and Use Committee Approval: 24/01/2011 №1

Application of flexible nanoholey patches for healing of infected skin wounds

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Keywords: Skin Infections, Staphylococcal; Infection, Wound; graphene oxide; photothermal therapy.

Introduction: The new approach to wound treatment by means of flexible nanoholey patches has proved to be an efficient alternative to antibiotics. The flexible skin patches together with the impact of a certain temperature destroy pathogens, resulting in quicker wound healing without possible negative post-effects of antibiotics.
Methods: Gold nanoholes modified Kaptons (K/Au NHs) and graphene coated K/Au NHs (K/Au NHs-rGO) were constructed in Univ. Lille, CNRS. The depilated skin of mice was infected by *S. epidermidis* bacterial cell culture containing 2×10^7 cfu mL^{-1} leading to superficial skin infection. After a period of 24 h, patches were applied to the skin. Each patch was illuminated for 5 min with a light emitting diode (LED) array (6×6 mm2 in size, 10 W, 940 nm). The skin was removed, photographed, and fixed for FFPE histology.
Results: To test the potential of K/Au NHs-rGO patches (1×2 cm^2 in size) in combination with the LED microarrays for the treatment of local skin infections. White laboratory Balb/c mice were infected and used for photothermal studies. After 5 days of infection initiation, superficial bacterial infiltrate was observed; however, in the place of contact with the patch no obvious infiltrate was detected. The images of the infected skin area obtained before and 72 h after the treatment (using the K/Au NHs-rGO patches) manifest clear and significant decrease of detected erythema compared to untreated as well as K/Au NHs treated wounds, thus indicating the definite advantage of this treatment procedure.
Discussion and Conclusion: The novel nanotechnology approach to the local early stage treatment of infected skin wounds was proved to be efficient and promising. It offers great potential for further development of local treatment strategies for infected wounds.

Source(s) of research support: The study was supported by the EU project H2020-MSCA-RISE-2015, PANG-690836.

Ethical Committee Approval: All animal experiments were approved by the Ethical Committee of DH LNMU (N8/18.09. 17).

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

Better Survival and Delayed Neuronal Stress After Stroke in Mice Lacking TLR2

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Keywords: ischemic stroke, TLR2, innate immunity, synaptic plasticity

Introduction: Postischemic inflammation includes deleterious mechanisms, which in turn increase the size of the lesion. However, by abolishing innate immunity, reactivation of plasticity is also postponed. To elucidate the triggers of those mechanisms, mice lacking Toll Like Receptor 2 (TLR2) were used in a model of stroke.
Methods: In order to monitor reactivation of Growth Associated Protein 43 (GAP43) after stroke induced by 60 minutes transient Medial Cerebral Artery Occlusion (tMCAO), *Tlr2* knock out mice (Tlr2-/-) were crossbred with *Gap43-luc/gfp* transgenics. Lesion size was imaged by high-resolution MRI (Bruker BioSpec 7T), while bioluminescence imaging (BLI) (IVIS, PerkinElmer) was monitored using free d-aminoluciferin and caged DEVD-luciferin. This strategy enabled assessment of recovery (GAP43) and neuronal stress marked by expression of caspase 3 (CASP3) – a protease responsible for release of caged DEVD-luciferin. Functional recovery was assessed by a battery of behavioural tests.
Results: Tlr2-/- mice had significantly better survival than their WT counterparts. Lesion size and its dynamics did not differ between the controls and Tlr2-/- mice, but there was a significant difference in BLI signal at chronic time points for GAP43 in mice lacking TLR2. CASP3 activity measured by DEVD-luciferin was also increased in Tlr2-/- mice, indicating higher neuronal stress represented by CASP3 activity. Tlr2-/- mice had milder onset of sensory impairment of the contralateral forelimb, and prolonged recovery in comparison to WT.
Discussion: Lack of TLR2 contributed to higher survival in mice after stroke, which subsequently led to longer recovery and higher CASP3 activation, then in their WT controls.
Conclusions: The post-stroke mortality due innate immunity was decreased by inhibition of the TLR2 pathway. However, careful modulation of this signaling is necessary in order to enable on-time regeneration.

Ethical approval: University of Zagreb, School of Medicine no.: 380-59-10106-17- 100/187.

Acknowledgements: This study was supported by the EU FP7 grant GlowBrain (REGPOT-2012-CT2012-316120). Multimodal imaging was done at Laboratory for Regenerative Neuroscience - GlowLab, University of Zagreb School of Medicine, Croatia.
We thank Cedars-Sinai Medical Center's International Research and Innovation in Medicine Program, the Association for Regional Cooperation in the Fields of Health, Science and Technology (RECOOP HST Association) for their support of our organization as participating Cedars – Sinai Medical Center - RECOOP Research Centers (CRRC).

Assessment of Preparation Protocols for Multimodal *ex vivo* Mouse Ischemic Brain Lesion Visualization

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Keywords: magnetic resonance imaging, synchrotron, ex vivo, brain, stroke

Introduction: *Ex vivo* imaging is preferred over *in vivo* for observing tissue architecture at higher resolutions, due to longer achievable scan times. Moreover, *ex vivo* tissue preparation involves dehydration and structural changes, which often reduce signal-to-noise (SNR) and contrast-to-noise (CNR) ratios. Another issue is the incompatibility of preparation protocols for different imaging modalities, such as using ethanol in MRI. In this study, we evaluate the cross-compatibility of tissue preparation protocols, in particular for MRI and CT.
Methods: C57Bl/6 albino mice underwent cerebral ischemia induced by 60-minute middle cerebral artery occlusion. In group 1, the isolated brains were dehydrated by Evaporation-of-Organic-Solvent (EOS) method, optimized for synchrotron radiation phase-contrast CT imaging (SRμCT), and afterwards rehydrated to PBS for MRI. In group 2, the brains were first fixed with 4% PFA, kept in PBS for MRI imaging, and subsequently treated by EOS for SRμCT.
SRμCT images were acquired using white beam radiation at an isotropic resolution of 2.35 um. MRI images were obtained with a T2-weighted sequence with a resolution of 0.1x0.1x0.4 mm.
Results: The contrast between the lesion area and surrounding tissue in MRI images was higher in group 1. Conversely, the morphological features of the brain were better preserved in group 2. The rehydration of EOS-prepared samples (group 1) did not reproduce the same level of SNR as in PBS samples (group 2), nor did the rehydrated brains regain their initial size after rehydration.
Discussion: The observed differences can be attributed to (EOS) tissue preparation, which removes both water and lipids from the tissue, affecting the myelin sheath structure. This translates to a change in T2 response in MRI, and affects mostly the myelin-abundant regions such as the corpus callosum.
Conclusions: EOS tissue preparation preserves sufficient contrast between the ischemic lesion and surrounding tissue for both MRI and SRμCT imaging. However, the SNR of EOS-prepared samples was rather low for MRI imaging.

Ethical approval: All experimental procedures were approved by the University of Zagreb, School of Medicine Ethics Committee (380-59-10106-17-100/187).
Acknowledgements: This work was supported by Croatian Science Foundation IP-06-2016-1892 RepairStroke project and by Synchrotron Light Source 'Elettra' grant n. 20170140. The MRI scans were performed at the Laboratory for Regenerative Neuroscience – GlowLab, University of Zagreb, Croatia.
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Activation of neutrophil extracellular traps under high-fat high-cholesterol diet

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Keywords: neutrophil extracellular traps; lithogenic diet; gallstones.

Introduction: In healthy conditions, neutrophils patrol the body's tissues and surface of organs. But, under pro-inflammatory conditions or after the contact with various crystals neutrophils externalize decondensed chromatin decorated with granular proteins producing neutrophil extracellular traps (NETs). Cholesterol nanocrystals were recently reported to induce NETs, and NETs were shown to oclude pancreatic ducts. Here we aimed to study effect of a high-cholesterol diet on NET formation in laboratory mice.
Methods: Mice weighing 25-35 g were kept on a lithogenic pellet diet containing 10% fat, 1% cholesterol and 0.5% cholic acid (Altromin GmbH) with free access to water. Neutrophil depletion of animals was achieved by i.p. injection of 1A8 antibodies, while isotype control 2A3 antibodies served as treatment control. The weight, and behavior of the animals were monitored. After 4 to 8 weeks of treatment animals were removed from the experiment, and protein, bilirubin and blood were measured in urine, gall bladders were surgically removed and analyzed for the presence of gallstones; and liver, gall bladder tissue were analyzed by FFPE histology with H&E for signs of liver damage. Obtained gallbladder smudges were analyzed for neutrophil components using IHC with PI, neutrophil-elastase and anti-citrullinated histone H3 antibodies.
Results: Under the condition of a high-fat high-cholesterol diet, we found gallstones in ~60% of mice. Lithogenic diet also led to the development of NASH (non-alcoholic steatohepatitis). H&E staining of the mice liver under the diet showed an obvious vacuolar degeneration coupled with neutrophil infiltration. IHC of gallstone material allowed us to decipher the role of neutrophils and NETs in the formation of gallstones.
Discussion and Conclusion: Based on our results we demonstrate that NETs are contributing to gallstone formation, development of liver steatosis and NASH (non-alcoholic steatohepatitis). The obtained data can be used to provide a more effective NASH prevention strategy.

Source(s) of research support: The study was supported by VW Stiftung Project No90361.

Ethical Committee Approval: The animal experiments were approved by the Ethical Committee of DH LNMU (N5/23.02. 17 & N2/15.02.16) and were conducted in accordance with the EU Council directives on laboratory animals (86/609/EEC).

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Obesity-provoked changes in bone morphogenetic protein and inhibitor of DNA-binding: levels in placenta of pregnant rats and blood plasma of pups

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Keywords: obesity, pregnancy, BMP/Smad signaling, Id proteins.

Introduction: Disproportionate growth of adipose tissue leads to excessive production of adipocytokines that are cell signaling proteins that change the activation of several signaling pathways that regulate development of organs and tissues, including bone morphogenetic proteins (BMPs) and their targeted genes. A deeper understanding of molecular mechanisms of the adverse effect of maternal obesity on pregnancy needs the generation of novel strategies for preventing and treating obesity-provoked developmental abnormalities.
Methods: Obesity in female SPRD rats was induced using a classical high fat high sugar diet protocol. The levels of BMP-2 in plasma of mums and newborn pups, phosphorylated Smad1/5/8, and the inhibitor of DNA-binding proteins (Id1 and Id2), direct BMP/Smad target genes, in placental tissue were detected by the ELISA and Western blot approaches.
Results: Obese rats have an increased maternal weight and a decreased weight of fetuses and placentas. Plasma level of BMP-2 was significantly higher in plasma of the newborn rats delivered by obese mothers. BMP-2 was below detection level in plasma of mothers on both the standard diet or high fat high sugar diet. We observed a tendency of increase in the level of phosphorylated Smad1/5/8, Id1 and Id2 proteins in placenta of obese mums.
Discussion: One can speculate that the revealed changes may affect the trophoblast lineage development and placental differentiation, the folding of the neural plate, MAPK signaling activation, and the formation of new blood vessels.
Conclusion: Obesity causes an increase in the BMP-2 level resulting in a tendency towards an elevated amount of the phosphorylated form of Smad1/5/8 and Id1/2 proteins in the placenta of obese rats. Obesity provokes fetal and placental growth retardation.

Ethical Committee or Institutional Animal Care and Use Committee Approval. All experiments with animal subjects were carried out in the Department of Pharmacodynamics and Biopharmacy, Faculty of Pharmacy, University of Szeged, Hungary with the approval of the Hungarian Ethical Committee for Animal Research (permission number: IV./3071/2016.).
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Cytotoxic Effect of Recombinant Analog of Lactaptin Rl2 is Associated with Enhanced ROS Production In Tumor Cells

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Keywords: recombinant analog of lactaptin, tumor cells, cytotoxic effect, reactive oxygen species

Introduction. Lactaptin is a proteolytic fragment of human milk kappa-casein (residues 57–134; m.m. ~8.6 kDa) capable of reducing viability of different tumor cells through inducing their apoptosis. Thus, it can serve as a basic molecule for development of new anticancer drugs. RL2 polypeptide is a prospective recombinant analog of lactaptin possessing an essential cytotoxic activity towards different tumor cells *in vitro* and *in vivo*. However, the potential molecular mechanisms of the antitumor activity of RL2 remain poorly studied. The action of many anticancer drugs is accompanied by the elevated production of the reactive oxygen species (ROS) via dissipation of the mitochondrial membrane potential in target cells. Here we hypothesize that the apoptotic effects of LR2 are linked to the production of ROS.
Methods. A suspension of human T-cell leukemia Jurkat cells and substrate-dependent human breast adenocarcinoma MCF-7 cells were used. The MTT and Trypan blue staining assays were applied for measuring a cytotoxic effect of RL2. Flow cytometry and fluorescent microscopy were used in order to evaluate the ROS level in target cells.
Results. We found that treatment of tumor cells of Jurkat and MCF-7 lines by RL2 polypeptide fragment of lactaptin led to a dose-dependent enhancement of ROS production in these tumor cells that correlated with their apoptosis.
Conclusion. The obtained data suggest that the RL2 polypeptide induces apoptosis in target tumor cells through direct or indirect effects on the mitochondria by stimulating ROS production.

Source of research support: This work was supported by a grant from the Volkswagen Shifting Trilateral Partnerships–Cooperation projects between scholars and scientists from Ukraine, Russian Federation and Germany.

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Lectins in the investigation of cardiac morphology and function

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Keywords: lectin histochemistry, human myocardium, post-infarction cardiosclerosis

Introduction. Lectin histochemistry methods are far from complete for investigations of the heart, remodelling of cardiac tissue carbohydrate determinants under different pathological conditions, including the progression of ischemic disease, post heart attack lesions, as well as possibilities for predicting post-infarction rehabilitation processes.
Methods. We investigated cardiac muscle samples, excised from left ventricular areas of 5 patients with post mortal diagnosis "post-infarction cardiosclerosis, recurring acute myocardial infarction". Carbohydrate determinants of glycoconjugates were detected using plant lectins: WGA, RCA, and LABA. Lectin receptor sites were visualized using diaminobenzidine tetrahydrochloride (Sigma, USA). Microscopy was conducted and pictures taken using a Granum R6053 photomicroscope equipped with Echoo-Imager.
Results. Post-infarction cardiosclerosis was accompanied by hypertrophy of cardiomyocytes in combination with numerous ruptures of muscle fibers with the defect replacement by connective tissue elements – signs of microfocal cardiosclerosis. Some contractile cardiomyocytes lost their nuclei, others exhibited signs of karyopyknosis and karyorhexis, encompassing apoptotic conditions in them. Lectin labels in myocardium samples were restricted predominantly to plasma membranes of erythrocytes and contractile cardiomyocytes, as well as to perinuclear granularity of the latters, responding to lipofuscin inclusions. It is noteworthy that lipofuscin granules could be visualized without lectin and even diaminobenzidine preincubation, therefore causing a false-positive reaction.
Discussion. In post-infarction cardiosclerotic lesions lectins distinctly labeled stromal elements, including the microvascular bed, and tissue detritus. WGA staining additionally demonstrated presence of atypical spindle-shaped endothelial cells within the blood vessels, exposed strong reactivity with cytoplasmic glycoconjugates of lymphocytes and plasma cells, and desorganized fibrous elements of perivascular localization. WGA and LABA lectins did not react with contractile cardiomyocyte nuclei, and myofibrils were not RCA-positive.
Conclusions. In comparison with the routine morphological methods, WGA and RCA demonstrated significantly higher selectivity of connective tissue and heart muscle microvascular bed labeling, therefore these same lectins can be recommended as an alternative for quantitative evaluation of cardiosclerotic lesions.
Acknowledgement: We thank Cedars-Sinai Medical Center's International Research and Innovation in Medicine Program, the Association for Regional Cooperation in the Fields of Health, Science and Technology (RECOOP HST Association) for their support of our organization as participating Cedars-Sinai Medical Center-RECOOP Research Centers (CRRC).
Ethical Committee or Institutional Animal Care and Use Committee Approval:
12/05/2017 № 4

Visegrad Grant Report

Final report on the research project (Visegrad Fund Scholarship No. 51701064)
" Obesity in pregnancy: the impacts of bone morphogenetic and inhibitor of DNA-binding proteins in rats"
of Nataliya Finiuk
for 1st semester (5 months) from September 18, 2017 to January 31, 2018

Obesity is a major health challenge in the world. It should be noted that obesity can lead to type 2 diabetes, atherosclerosis, cancer, thus leading to increased mortality (Svendstrup and Vestergaard, 2014). Adipose tissue expansion depends on new blood vessel formation in order to prevent hypoxia and tissue destruction causing inflammation.

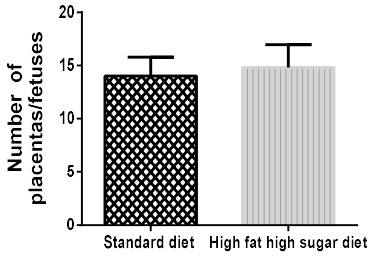
Proper development of the embryo after implantation depends on the formation and appropriate functioning of placenta (Liang et al., 2016). Placental malfunctions induce pregnancy disorders which contribute to life-threatening complications for both mother and fetus. Identification and characterization of crucially important signaling pathways' interactions in both placenta and fetus is required for deeper understanding of the processes that control placental function and development of embryo.

Excessive development of adipose tissue leads to production of several signaling molecules implicated in the regulation of organs and tissues development. These include fibroblast growth factors, wingless, nodal, members of the transforming growth factor (TGF)-β family, including bone morphogenetic proteins.

A deeper understanding of the molecular mechanisms underlaying the adverse effect of mothers' obesity on the pregnancy is desperately needed for generation of novel strategies in preventing and treating of obesity-provoked developmental abnormalities.

Methods: Study was performed with female rats. Obesity in experimental animals was induced using classical high fat high sugar diet protocol. The expression levels of BMP2, phosphoSmad1/5/8, Id1 and Id2 proteins in plasma of mums and newborn pups as well as placental tissue were detected by ELISA and Western blot approaches. All experiments with animal subjects were carried out with the approval of the Hungarian Ethical Committee for Animal Research (Department of Pharmacodynamics and Biopharmacy, Faculty of Pharmacy, University of Szeged, Hungary).

Results: We found that obese (high fat high sugar diet (HFHSD)) rats have an increased maternal weight and a decreased weight of fetuses and placentas (fig. 1).



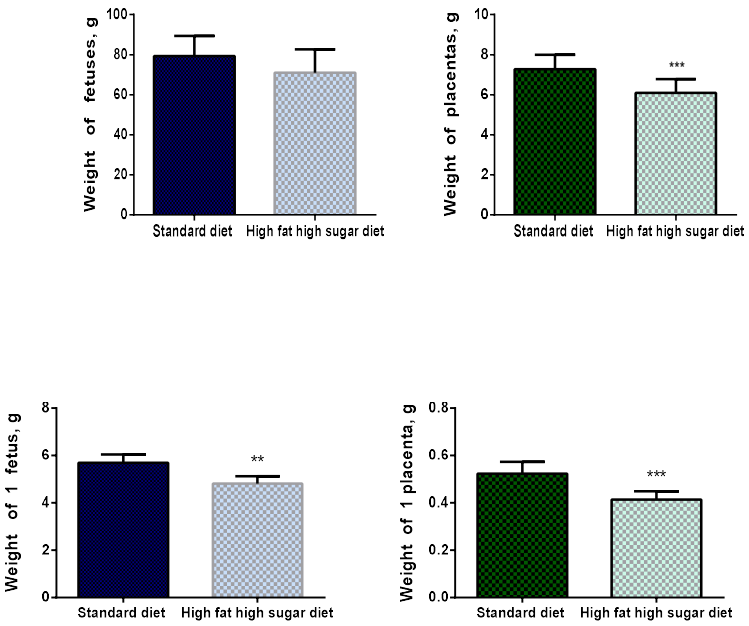


Fig. 1. Obesity effected weight of fetuses and placentas.

The ELISA assay was performed to investigate a BMP-2 level in plasma of newborn rats and mothers of standard diet and obese (high fat high sugar diet) rats.

Bone morphogenetic proteins (BMPs) regulate many processes through the embryonic development as well as in the maintenance of normal tissue function later in adulthood. BMP-2 is a cytokine that promotes bone formation and also is associated with potential adverse clinical effects (Ribeiro et al., 2017). Bone morphogenetic proteins 2 and 4 also regulate trophoblast lineage development and differentiation (Pennington and Ealy, 2012).

Plasma level of BMP-2 was significantly higher in plasma of newborn rats delivered by obese mothers (fig. 2). BMP-2 was below detection level in the plasma of mothers of both standard diet and high fat high sugar diet.

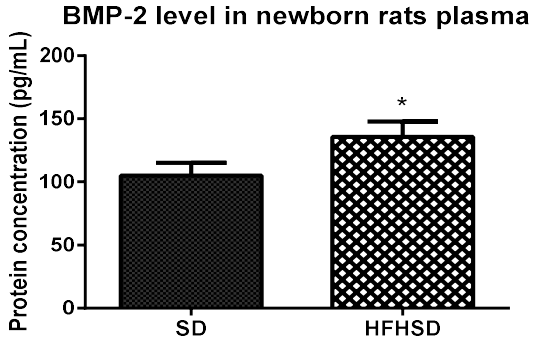


Fig. 2. BMP-2 level in plasma samples of newborn rats of standard diet (SD) and high fat high sugar diet (HFHSD).

The expression levels of phosphorylated Smad1/5/8 and BMP/Smad target genes Id1 and Id2 proteins in placenta samples from standard diet and high fat high sugar diet rats were investigated using Western-blot analysis on the 22 day of rat pregnancy.

BMP proteins mediate their effects via phosphorylation of Smad1/5/8, the so called BMP R-Smads (receptor-activated Smads) (Schulz and Tseng, 2009). Specific BMP receptors and mediators of their signals, Smad proteins are also involved into the proliferation, differentiation, and apoptosis regulation of various types of cells and organs not only during embryonic development but also in postnatal period (Huang et al., 2009; Burstyn-Cohen et al., 2004; Sela-Donenfeld and Kalcheim, 1999). Folding of neural plate is connected with a two-dimentionally spatiotemporal gradient of phosphorylated Smad1/5/8 (Phospho-Smad1/5/8) (Eom et al., 2011).

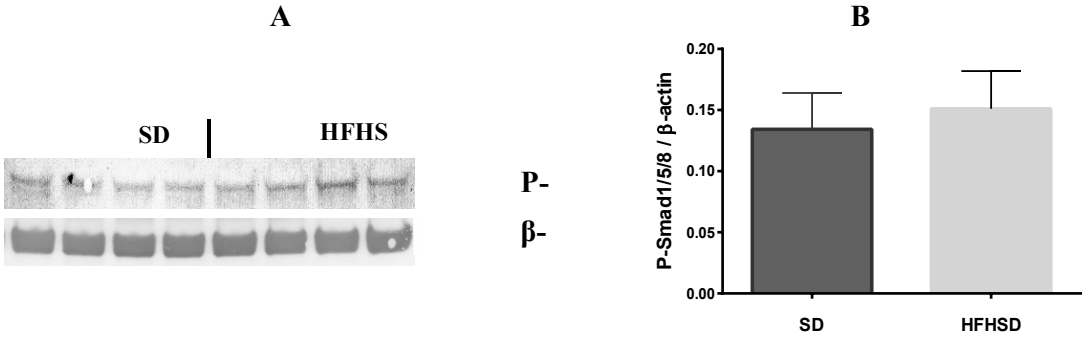


Fig. 3. The Western-blot analysis results of **phosphorylated Smad1/5/8** (P-Smad) expression level in placenta samples of standard diet and high fat high sugar diet rats: A – Western-blot analysis results; B – densitometry of protein level.

It was reported that BMPs can also activate the p38MAPK signaling cascade (Hata et al., 2003; Schulz et al., 2003) and/or extracellular signal-related kinase (ERK), the c-Jun N-terminal kinase (JNK) (Schulz et al., 2003).

We have observed a tendency to increase in the level of phosphorylated Smad1/5/8 protein in placenta of obese mums, but such changes were not-significant (fig. 3).

Id proteins (Id1, 2,3 and 4), the inhibitors of DNA binding, function as dominant-negative regulators of HLH transcription factors (Norton, 2000). Id proteins are direct BMP-Smad target genes (Korchynskyi and ten Dijke, 2002; Miyazono and Miyazawa, 2002; Yang et al., 2013; Roschger and Cabrele, 2017). Id proteins are required to preserve stem cell identity and prevent premature differentiation. Id proteins are widely expressed throughout development and function in the determination of cell specification into specialized lineages. In particular, Id2 is known to be an important regulator of placental differentiation, and protein are the highest in proliferative trophoblast stem cells and decline during differentiation into lineage-specific trophoblast subtypes in both humans and rodents (Selesniemi et al., 2016).

We have found a tendency to increase in the level of Id1 and Id2 proteins in placenta of obese mums, but such changes were not-significant (fig. 4).

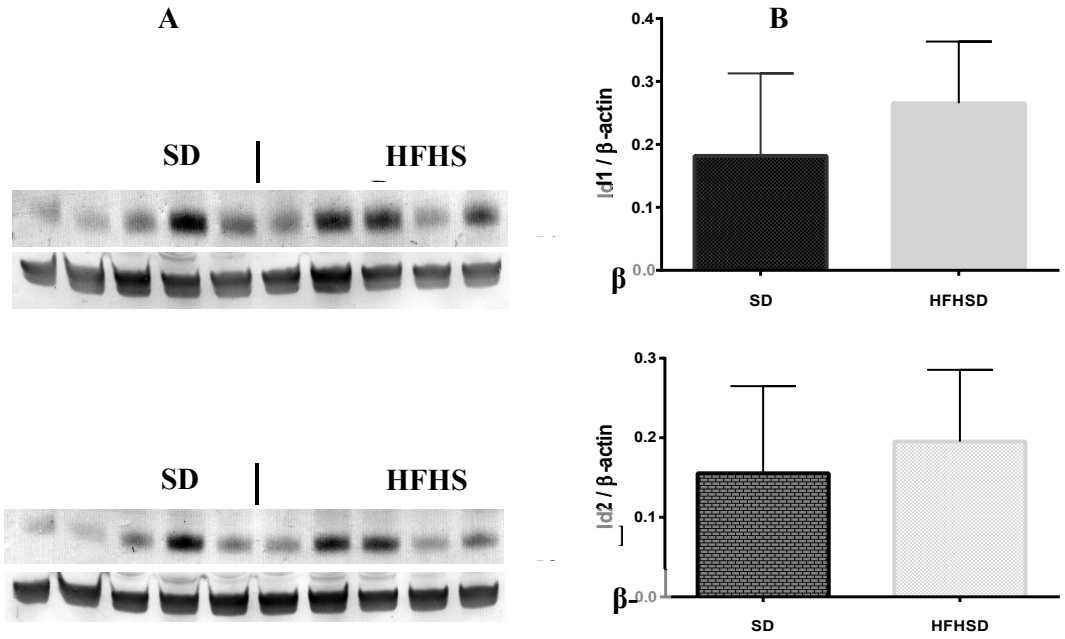


Fig. 4. The Western-blot analysis results of **Id1** and **Id2** expression level in placenta samples of standard diet (SD) and high fat high sugar diet (HFHSD) rats: A – Western-blot analysis results; B – densitometry of protein level.

In conclusion: we found increased level of bone morphogenetic protein 2 (BMP-2) and a tendency to increased levels of Id1 and 2 proteins and phosphorylated form of Smad1/5/8 protein in placenta of obese rats. Obesity caused fetal and placenta growth retardation. We can speculate that these changes may affect the trophoblast lineage development (Pennington and Ealy, 2012); the folding of neural plate, p38MAPK, ERK, JNK activation (Schulz et al., 2003) via BMP/Smad signaling and modulate the stress response factors (Roschger and Cabrele, 2017). It should be noted that high level of Id proteins could be found in highly proliferating cells (Billestrup et al., 2011). The dysregulation of Id proteins may induce cell-cycle arrest via Cdk inhibitors (p16, p21), pRb and p53 (Roschger and Cabrele, 2017). Id2, that affects the pRb pathway, may activate cell division and may trigger tumorigenesis (Chong et al., 2009). The increasing of Id1 may negatively effect the p53-mediated response to DNA damage (Qian and Chen, 2008). We can speculate that obesity may effect the placental differentiation, since sustained Id1 and Id2 expression can prevent differentiation into giant cells and extravillous trophoblasts (Selesniemi et al., 2016). The elevated Id1/Id3 expression was reported in β cells in the islets of Langerhans of diabetic mice (Wice et al., 2001; Busch et al., 2002; Billestrup, 2011) that indicates the Id proteins role in insulin secretion and energy metabolism. Dysregulation of Id1 protein may also affect the formation of new blood vessels. Id1 and Id3 are required also for neurogenesis, angiogenesis and vascularization of tumor xenografts (Lyden et al., 1999; Roschger and Cabrele, 2017). Id proteins were found to stimulate neural precursor cells proliferation while inhibit their differentiation (Andres-Barquin et al., 2000; Evans et al., 1993). In addition, Id2 might be important for the development of midbrain dopaminergic neurons in Parkinson's disease (Havrda et al., 2008; Konishi et al., 2010). In addition, the elevated Id2 expression may lead to the increasing of proliferation and self-renewal of glioma stem-like cells (Paolella et al., 2011).

Thus, BMP, Smad and Id proteins are important regulators during early development and also implicated as direct causes of defects in bone formation, angiogenesis, tumor formation and neurodevelopmental disorders.

This project, supported by the International Visegrad Fund, provided the opportunity to establish a productive cooperation between Department of Regulation of Cell Proliferation and Apoptosis, Institute of Cell Biology of National Academy of Sciences of Ukraine and Department of Pharmacodynamics and Biopharmacy, Faculty of Pharmacy, University of Szeged, Hungary. Both Institutes are Cedars – RECOOP Research Centers (CRRCs) as members of the RECOOP HST Association.
The RECOOP HST Association provided the matching fund of 1,500 USD for wet laboratory reagents to support the completion of our research.

This work helped me to learn the new techniques and form new of personal qualities. I built new professional network and formed plan for future scientific research projects based on the obtained results. It should be mentioned that this project was an important stage in my development as independent scientist.

We are going to present obtained results on RECOOP 13th Bridges in Life Science Annual Conference on April 12 – 15, 2018, Zagreb, Croatia.

RECOOP Visegrad Scholarship Program

Visegrad Scholarship http://visegradfund.org/scholarships/

The top ten young scientists selected during the Bridges in Life Sciences Annual Conferences may apply for International Visegrad Fund (IVF) Scholarship and receive the RECOOP Young Scientists Matching Fund. The Visegrad Scholarship is the Visegrad Four European Macro-Region's Fulbright Program. Therefore, it could be important to link the Visegrad Scholarship and the Fulbright Foreign Student Program.

Visegrad Scholarship Program (VSP)

The International Visegrad Fund offers Master's and Post-Master's scholarships awarded to selected scholars for periods of 1 or 2 semesters (with the exception of Master's scholarships within the Visegrad Scholarship schemes where 1– to 4-semester scholarships can be awarded).

The following scholarship schemes are available:

Intra-Visegrad Scholarships
In-Coming Scholarships
Out-Going Scholarships
Scholarship Program for Belarusian Students
Scholarship Program for Ukrainian Students
Visegrad Scholarships at OSA Archivum (separate program)

If selected each scholar receives the scholarship funding at the beginning of each five-month period (semester) upon a written confirmation from the host university/institution.
Deadline for all scholarship applications is **31 January**. Results are announced by mid-May.

CSMC – RECOOP Research Centers (CRRC) the Center of Excellences of the RECOOP HST Association. They host young scientists, Ph.D. students with CSMC – RECOOP (IVF – CSMC - RECOOP) Scholarship. The RECOOP HST Association Scientific Advisory Board selects the young scientists who could compete for IVF – CSMC - RECOOP Scholarship. The selected young scientists (preferably Ph.D. students) will spend a maximum of four semesters at the host organization and receive: €2,300 / semester and the corresponding host universities/institutes receive €1,500/semester/scholar. The host CRRC will get $1,500 for laboratory expense and consumables from CSMC – RECOOP HST Association. Applicants whose current (i.e. at the time of applying) university or employer is further than 1,500 km from the selected host university/institute are eligible for a one-time travel grant.

RECOOP HST Association Members from the Visegrad Group Countries:
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University of Szeged, Hungary
Slovak Medical University, Bratislava, Slovakia
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RECOOP HST Association's Cedars – RECOOP Research Center (CRRC) could participate
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